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TECHNIQUE FOR DETERMINATION OF THE ANTINEURITIC VITAMIN B[1]

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IN THE studies of vitamin B reported in the literature, we find that various workers report different quantities of yeast necessary for what they term normal growth. These quantities range from .05–.7 gm daily. Some of these differences can be related to the potencies of the yeasts used. However, we feel that many of these differences are due to the composition of the basal rations used. An examination of the literature at once reveals that there is no uniformity in the character of the basal rations used for vitamin B measurements. Some workers use starch exclusively as their source of energy; some use starch and fat, while others employ sucrose in their dietary mixtures.

We have had occasion in this laboratory to make a great many measurements of materials for their antineuritic vitamin B content. Autoclaved yeast was present in all our diets, so that we were working as far as we know with only one of the vitamin B factors. We have found that the growth obtained in the absence of the antineuritic vitamin B depends largely upon the basal diet used. The following factors have been found to be of importance: (1) The amount of protein, (2) the kind and amounts of carbohydrates used, (3) amount of fat. The last named is of particular importance.[2]

Figure 1 shows the growth on diets, *devoid of any added antineuritic B* but varying in the form of energy or amount of protein that they carry. The poorest growth is obtained when the diet consists of sucrose and 25 per cent protein[3] (Diet C). When 10 per cent of fat is added to this

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, the United States Bureau of Dairying, and the School of Agriculture and Board of Research of this institution.

[2] Evans, Herbert M. and Lepkovsky, S. Sparing action of fat on the antineuritic vitamin.—*Science*, September 28, 1928, LXVIII, 298.

[3] All references to protein include the extracted casein (L−3) plus the protein furnished by the 10 per cent autoclaved yeast. Approximately 50 per cent of the yeast is protein.

diet replacing an equal amount of sucrose (Diet E) little or no improvement results. If the sucrose is replaced with dextrinized corn starch (Diet D) slight growth improvement results. When 10 per cent of the starch is replaced with an equal amount of fat (Diet F), further improvement in

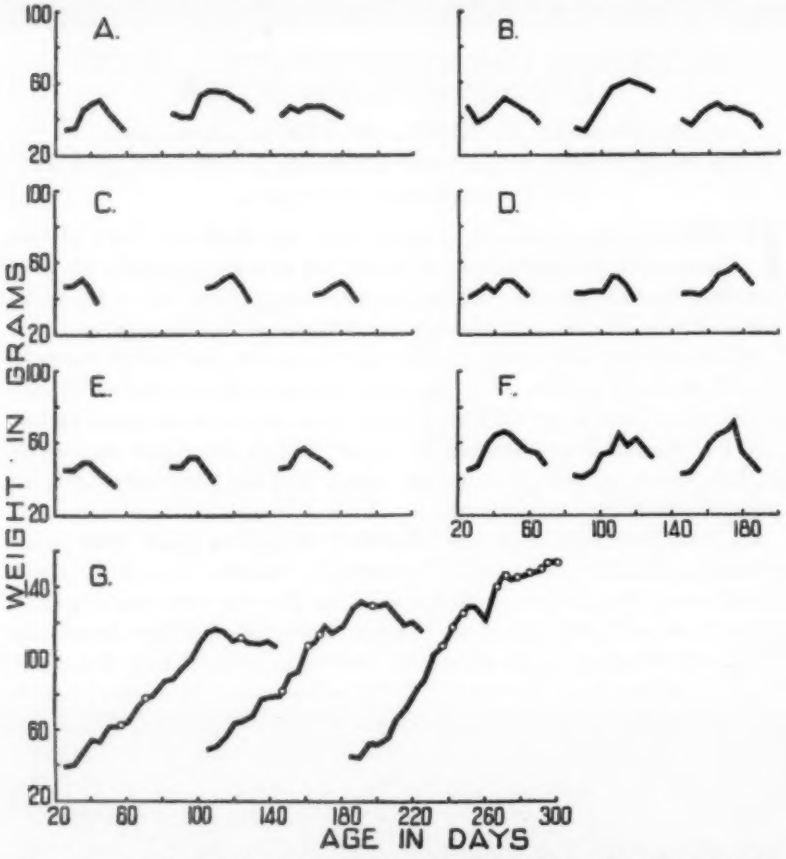

FIG. 1.—Depicts the growth of animals on diets without any added antineuritic vitamin B.

growth results. If the protein content is raised to 50 per cent (Diet A), better growth is obtained than on the diet with 25 per cent protein (Diet C), and when the amount of the protein is raised still further (75 per cent of the diet—Diet B) a slightly greater improvement in growth results. If a high per cent of fat is used with the protein (Diet G), moderately good growth is obtained without any added antineuritic vitamin B.

The animals on this diet remained in fairly good condition for six months, but one is now showing typical symptoms of beri-beri.[4]

Figure 2 shows the same diets *with 50 mg. brewers' yeast added as a low sources of the antineuritic vitamin B.* The poorest growth here is

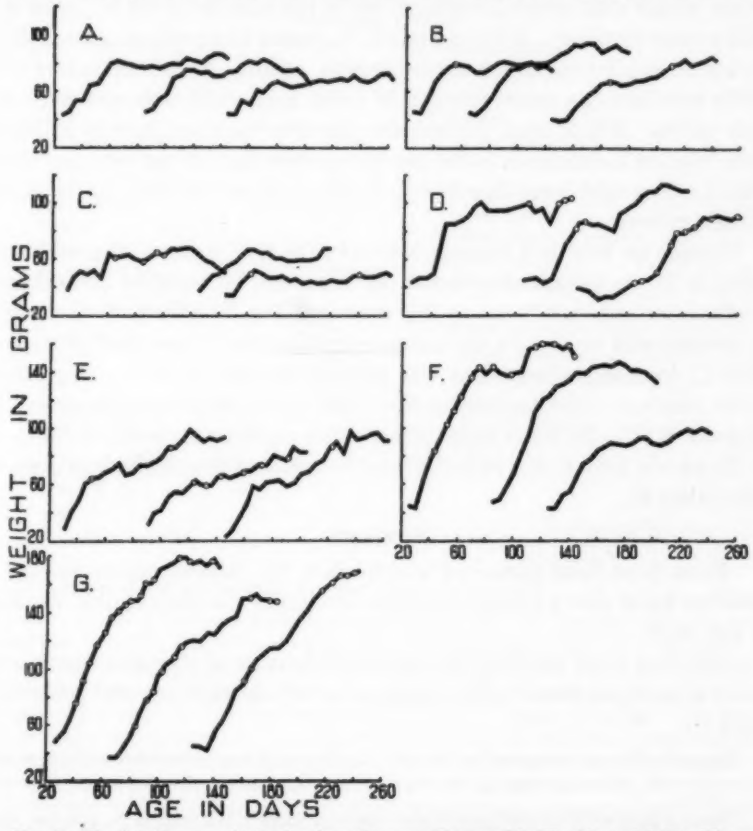

FIG. 2.—Depicts the growth of animals on the same diets used in Fig. 1 but with 50 milligrams of brewers' yeast added as a low source of antineuritic vitamin B.

again obtained on the sucrose-protein diet (25 per cent protein—Diet C). On this diet, the animals plateau at about 80 grams in weight and then slowly decline until death intervenes, usually with symptoms of beri-beri.

[4] The symptoms of beri-beri as seen on diets low in the antineuritic vitamin B may be described as follows: The hind limbs especially are spastic and are lifted in a stiff, high way. There are extensor thrusts of the fore and hind limbs. Movements in a circle are often observed. Ultimately paralysis of the legs supervenes.

If 10 per cent fat replaces an equal amount of sucrose (Diet E), growth is considerably accelerated. In the presence of 50 per cent fat (Diet G) very close to normal performance is observed. When corn starch replaces sucrose (Diet D) as a source of energy there is improvement in growth. When 10 per cent starch is replaced by 10 per cent fat (Diet F) there is a still greater response. If the protein is increased in a protein-sucrose diet, (Diets A and B) improvement in growth results. From these data it is easily seen how the same amount of yeast may yield different antineuritic values. It is at once evident that the diet most sensitive to antineuritic vitamin B deficiency is the sucrose-protein diet (25 per cent protein—Diet C). It would seem, therefore, to be the most suitable diet for the study of this vitamin.

Though we were not directly interested in the "balance of proteins to vitamin B" so extensively studied by Hartwell, Drummond and others,[5] we find that our data bear on this question (Fig. 2). When the amounts of protein and sucrose in our sucrose-protein diet (25 per cent protein—Diet C) were interchanged as was the case in Diet B, marked improvement resulted, thus indicating that added protein acted as a sparer of vitamin B.[6] On the other hand, when starch replaced sucrose in Diet C, as is the case in Diet D, the improvement was greater than in the high protein diet (Diet B).

Summary

Data have been presented which show the importance of having a uniform basal diet when comparative studies of the antineuritic vitamin B are made.

The diet most sensitive for the determination of the antineuritic vitamin B has been found to be a sucrose-protein diet (25 per cent protein—Diet C).

The curves illustrate the growth of individual female rats. Litter mate sisters were distributed throughout the various groups. All the animals were placed on the diets at 21 days of age and

[5] Most of these studies were done with the vitamin B complex; Hartwell, G. A., *Biochem. Jour.* XVIII, 785 (1924); Reader, V. and Drummond, J. C., *Biochem. Jour.* XX, 1256 (1926); Sherman (*Jour. Biol. Chem.* LXXIV, 117 (1927)) writes "no basis is found for any belief that the vitamin B requirement is influenced by the protein intake in the age period covered by these experiments."

[6] This interpretation of the data must be taken with due caution. Though our casein was washed thoroughly to remove antineuritic vitamin B, we cannot be certain that this has been entirely accomplished (Palmer, L. S. and Kennedy, C., *Jour. Biol. Chem.* LXXIV, 591 (1927)). and hence improvement may be due to unextracted B residual in the casein. Our experience with our basal sucrose-protein diet (25 per cent protein—Diet C), either does not admit the possibility of residual antineuritic B in our casein, or, if such minute impurities do exist, they are evidently without noticeable influence in our studies. Furthermore, it should be emphasized that we worked only with one level of antineuritic vitamin B (50 mg. yeast) and we have no reason to believe that our observations at this level will necessarily hold for other levels of the vitamin.

maintained in cages with wire mesh floors. The animals were watched daily for breakdown of the vaginal closing membrane and thereafter for oestrous changes in the vaginal smear indicative of ovulation. The small circles interrupting the growth curves in the charts indicate times of ovulation.

COMPONENTS OF THE DIETS USED IN THE EXPERIMENTS REPRESENTED IN FIGURES 1 AND 2.

A—*Sucrose-Protein 50%*

Casein (L-3)	45
Sucrose	45
Salts	4
Autoclaved yeast	10

B—*Sucrose-Protein 75%*

Casein (L-3)	70
Sucrose	20
Salts	4
Autoclaved yeast	10

C—*Sucrose-Protein 25%*

Casein (L-3)	20
Sucrose	70
Salts	4
Autoclaved yeast	10

D—*Starch-Protein 25%*

Casein (L-3)	19
Dextrinized cornstarch	67
Salts	4
Autoclaved yeast	10

E—*Sucrose-Lard-Protein 24%*

Casein (L-3)	20
Sucrose	59
Salts	4
Autoclaved yeast	10
Lard	10

F—*Starch-Lard-Protein 24%*

Casein (L-3)	20
Dextrinized cornstarch	59
Salts	4
Autoclaved yeast	10
Lard	10

G—*Lard-Protein 41%*

Casein (L-3)	36
Salts	4
Autoclaved yeast	10
Lard	50

All diets were supplemented with two drops of cod liver oil daily.

Casein (L—3)—Commercial casein (Golden State Milk Products Company) which has been precipitated from milk with hydrochloric acid, dried and delivered to us in sacks, was extracted in wooden tubs with acidulated water (40 cc. glacial acetic acid to 40 liters of filtered tap water). The acidulated water was siphoned off and replaced twice daily for six days. On the seventh day the casein was washed twice with distilled water replacing the acidulated tap water. The water was thoroughly drained from the casein and the casein washed once with 50–80 per cent alcohol and once with 85–95 per cent alcohol followed by one ether treatment. It was then spread in pans and the ether allowed to dissipate at room temperature under a fan.

Autoclaved yeast—Whole dried yeast (bakers') was generously supplied us by the Fleischmann Company of New York. It was spread in pans to a depth of less than one inch and autoclaved for five hours at 18 to 20 pounds pressure. This yeast was added as the source of the thermostable water-soluble factor throughout the experiment. Abundant trials showed it to be almost entirely, if not absolutely, free from antineuritic B.

Brewers' yeast—A whole, dried brewers' yeast of high potency was used as the source of the thermolabile or antineuritic factor B. It was generously furnished by the Vitamin Food Company through Dr. Edward A. Rumley of New York.

Dextrinized corn starch was prepared by pouring cold starch paste into boiling water, boiling for 5 minutes, spreading in thin layers in aluminum pans and drying at 110–120° C.

Salt mixture #185 after McCollum—Sodium chloride 51.0, crystals of magnesium sulphate 159.6 monobasic sodium phosphate 104.1, monobasic calcium phosphate 162.0, dibasic potassium phosphate 286.2, ferric citrate 35.4 and calcium lactate 390.0.

A STUDY OF THE EFFECTS OF CERTAIN DIETS UPON THE GROWTH AND FORM OF ALBINO RATS

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MUCH study has been given in the past to the effects of deficient diets on the skeletal growth of the animal body. Jackson (1924) has reviewed the work that has been accomplished along this line up to 1924, while more recently Winters, Smith and Mendel (1927) have published the results of a study of the effects on growth of animals of a low calorie diet, of inadequate protein, and of inadequate salt intake. No reference, however, has been found regarding the effect of a vitamin deficiency on general skeletal growth.

One of the purposes of the present investigation was to study the effects of a definiency of vitamin A or vitamin B upon the development of the body of albino rats, the specific purpose being to determine in what way the girth of the chest, the width of the hips and the size of the long bones of the legs of such animals compared with corresponding measurements of normal animals of the same age. The term vitamin B as used in this paper covers both the antineuritic substance and the more heat-stable substance now known as the antipellagric vitamin (vitamin B_2 or G).

The normal rats used for comparative purposes in this study were from diet No. 13 (Diet B), consisting of one-third whole milk powder, two-thirds whole wheat and sodium chloride two per cent of the wheat.

At the time the investigation was started there were available rats from another adequate diet, No. 16 (Diet A) consisting of one-sixth whole milk powder, five-sixths whole wheat and sodium chloride two per cent of the wheat. Although the animals receiving Diet A were apparently normal, they differed somewhat from those receiving Diet B. Earlier experiments (Sherman and Campbell 1924) have shown the latter animals to be considerably heavier at any given age, to have a longer life-span, to have a better reproduction record, and to be less subject to lung infection. A study of the calcium and phosphorus content (Sherman and MacLeod 1925), (Sherman and Quinn 1926) of rats from these two diets has also shown retardation of calcification in the animals on diet 16 (Diet A) during

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TABLE I

CERTAIN BODY AND BONE MEASUREMENTS OF ALBINO RATS ON ADEQUATE AND VITAMIN DEFICIENT DIETS

MALES

Diet No.	Average Age days	Maximum Weight Average grams	Final Weight Average grams	Trunk Length Average cm.	Chest Girth Average cm.	Femur		Tibia		Hip Width Average cm.	Humerus		Radius Length Average cm.	No. Animals
						Length Avg. cm.	Dia. Avg. cm.	Length Avg. cm.	Dia. Avg. cm.		Length Avg. cm.	Dia. Avg. cm.		
	29	47	47	12.2	7.4	1.86	.20	2.16	.15	.98	1.57	.16	1.47	10
	61	135	135	17.2	8.7	2.66	.25	3.03	.19	1.44	2.11	.20	1.96	13
13	95	226	226	20.3	11.3	3.15	.28	3.45	.21	1.68	2.48	.21	2.27	7
	132	272	272	21.7	11.7	3.44	.30	3.82	.21	1.81	2.70	.22	2.42	8
	225	295	295	22.6	12.8	3.61	.32	3.93	.22	1.96	2.86	.23	2.53	11
	426	292	284	22.6	12.8	3.61	.32	4.00	.24	1.94	2.88	.23	2.61	21
	30	42	42	11.7	5.9	1.80	.19	2.16	.14	.88	1.52	.16	1.45	6
	60	105	105	15.4	7.6	2.40	.22	2.75	.18	1.22	1.95	.19	1.78	7
	95	197	197	19.2	10.7	3.01	.28	3.27	.20	1.58	2.30	.20	2.14	12
16	130	235	235	20.7	13.3	3.29	.29	3.61	.23	1.73	2.55	.21	2.33	10
	224	273	273	21.7	12.9	3.51	.31	3.78	.22	1.87	2.74	.21	2.44	13
	473	275	226	21.6	11.9	3.45	.32	3.71	.24	1.86	2.69	.23	2.45	18
B	84	76	64	15.4	6.4	2.50	.23	2.90	.18	1.21	1.99	.16	1.80	18
107 B′	84	97	96	16.7	7.5	2.65	.25	3.06	.18	1.31	2.10	.16	1.90	26
A	73	104	75	15.9	6.6	2.63	.25	3.03	.19	1.29	2.05	.20	1.90	5
379a A′	104	109	108	16.6	7.9	2.67	.24	3.10	.19	1.29	2.08	.20	1.94	24

TABLE I (*continued*)

FEMALES

	30	43	43	12.0	6.3	1.86	.19	2.17	.15	.92	1.58	.17	1.45	7
	60	111	111	16.4	8.2	2.58	.23	2.91	.19	1.39	2.04	.19	1.88	9
13	99	199	199	19.4	10.5	2.99	.29	3.30	.20	1.61	2.40	.20	2.18	2
	212	215	215	20.2	12.4	3.25	.30	3.57	.21	1.80	2.53	.21	2.36	8
	444	217	214	20.5	11.8	3.30	.30	3.65	.22	1.75	2.62	.21	2.45	35
	30	41	41	11.4	5.5	1.75	.19	2.11	.13	.88	1.50	.16	1.43	5
	60	89	89	15.1	7.4	2.29	.20	2.54	.16	1.20	1.81	.18	1.61	3
16	94	152	152	17.7	9.6	2.86	.23	3.11	.20	1.48	2.22	.19	2.07	4
	184	193	193	19.1	13.0	3.17	.29	3.40	.24	1.68	2.43	.22	2.23	2
	478	207	194	19.6	11.8	3.19	.30	3.40	.23	1.67	2.45	.22	2.31	22
B	84	71	57	14.9	6.1	2.42	.23	2.81	.18	1.17	1.95	.15	1.78	16
107 B′	84	91	90	16.2	7.5	2.57	.23	2.95	.18	1.28	2.05	.16	1.88	18
A	84	88	66	15.1	5.9	2.49	.22	2.92	.17	1.17	1.91	.19	1.86	3
379a A′	105	98	97	15.9	7.7	2.57	.24	2.95	.19	1.22	2.01	.19	1.88	22

B—Animals in this group made average gains in weight of 6–11 grams during an 8 weeks' experimental period. B′—Animals in this group made average gains in weight of 50–55 grams during an 8 weeks' experimental period.

A—Animals in this group received from weaning or 28 days of age until their death the vitamin A deficient diet only. A′—Animals in this group made average gains in weight of 22–30 grams during an 8 weeks' experimental period.

TABLE II

AVERAGE RATIOS OF CERTAIN BODY MEASUREMENTS—ALBINO RATS ON ADEQUATE AND VITAMIN DEFICIENT DIETS.

Average Age days	Average max. weight grams	Weig't / Trunk Len'th	Weight / Chest Girth	Weight / Femur Length	Weight / Tibia Length	Weight / Hip Width	Weight / Humerus Length	Weight / Radius Length	Trunk Length / Chest Girth	Trunk Length / Femur Length	Trunk Length / Tibia Length	Trunk Length / Hip Width	Trunk Length / Humerus Length	Trunk Length / Radius Length	Femur Length / Femur Dia.	Tibia Length / Tibia Dia.	Humerus Length / Humerus Dia.	No. Animals
(Diet No. 13)																		
29	47	3.9	6.4	25.3	21.7	48.0	30.0	32.0	1.65	6.56	5.65	12.4	7.77	8.30	9.3	14.4	9.8	10
61	135	7.9	15.5	50.8	44.6	93.8	64.0	68.8	1.98	6.47	5.68	11.9	8.15	8.78	10.6	15.9	10.6	13
94	226	11.1	20.0	71.7	65.5	135	91.1	100	1.80	6.44	5.88	12.1	8.19	8.94	11.3	16.4	11.8	7
132	272	12.5	23.2	79.1	71.2	150	101	112	1.86	6.31	5.68	12.0	8.04	8.97	11.5	18.2	12.3	8
225	295	13.2	23.0	81.7	75.1	151	103	117	1.77	6.26	5.75	11.5	7.90	8.93	11.3	17.9	12.4	11
426	292[1]	12.9	22.8	80.9	73.0	151	101	112	1.76	6.26	5.65	11.6	7.85	8.66	11.3	16.7	12.5	21
(Diet No. 16)																		
30	42	3.6	7.2	23.3	19.4	48.0	27.0	29.0	2.02	6.50	5.42	13.3	7.65	8.07	9.5	15.4	9.6	6
60	105	6.8	13.8	43.8	38.2	86.1	53.8	59.0	2.03	6.42	5.60	12.6	7.90	8.65	10.9	15.3	10.3	7
95	197	10.3	18.4	65.4	60.2	125	85.7	92.1	1.79	6.38	5.87	12.2	8.35	8.97	10.8	16.4	11.5	12
130	235	11.3	17.7	71.4	65.1	136	91.8	101	1.56	6.29	5.73	12.0	8.12	8.88	11.3	15.7	12.1	10
235	273	12.6	21.2	77.8	72.2	146	99.6	112	1.68	6.18	5.74	11.6	7.92	8.89	11.3	17.2	13.1	13
473	275[2]	12.7	23.1	79.7	74.1	148	102	112	1.82	6.26	5.82	11.6	8.03	8.82	10.8	15.5	11.7	18
(Diet No. 107)																		
84	76[3]	4.9	11.9	30.4	26.2	62.8	38.2	42.2	2.41	6.16	5.31	12.7	7.74	8.56	10.9	16.1	12.4	18
84	97	5.8	12.9	36.6	31.7	74.0	46.2	51.1	2.23	6.30	5.46	12.8	7.95	8.79	11.5	17.0	13.1	26
(Diet No. 379a)																		
73	104[4]	6.5	15.8	39.5	34.3	80.6	50.5	54.5	2.41	6.05	5.25	12.3	7.77	8.32	10.5	15.9	10.3	5
104	109	6.6	13.8	40.8	35.2	84.5	52.4	56.2	2.10	6.21	5.35	12.9	7.98	8.56	11.1	16.3	10.4	24

TABLE II (*continued*)

FEMALES

(Diet No. 13)

30	43	3.6	6.8	23.1	19.8	46.7	27.2	29.7	1.90	6.45	5.52	13.0	7.59	8.22	9.78	14.5	9.29	7
60	111	6.7	13.5	43.2	38.1	79.9	54.4	59.0	2.00	6.36	5.63	11.8	8.04	8.72	11.2	15.3	10.7	9
99	199	10.3	19.0	66.6	60.3	124	82.9	91.3	1.85	6.49	5.87	12.0	8.08	8.89	10.3	16.5	12.0	2
212	215	10.7	17.3	66.2	60.1	119	84.9	91.1	1.61	6.21	5.65	11.3	7.98	8.56	10.8	17.0	12.1	8
444	217	10.6	18.4	65.8	59.5	124	82.8	88.6	1.74	6.21	5.62	11.7	7.82	8.37	11.0	16.6	12.5	35

(Diet No. 16)

30	41	3.6	7.5	23.4	19.4	46.6	27.3	28.7	2.07	6.51	5.40	13.0	7.60	7.97	9.21	16.2	9.38	5
60	89	5.9	12.0	38.9	35.0	74.1	49.2	55.3	2.04	6.59	5.94	12.6	8.34	9.38	11.5	15.9	10.1	3
94	152	8.6	15.8	53.1	48.9	103	68.5	73.4	1.84	6.19	5.69	12.0	7.97	8.55	12.4	15.5	11.7	4
184	193	10.1	14.8	60.9	56.8	115	79.4	86.5	1.47	6.03	5.62	11.4	7.86	8.57	10.9	14.2	11.0	2
478	207[5]	10.6	17.5	64.9	60.8	124	84.4	89.6	1.66	6.14	5.76	11.7	8.00	8.48	10.6	14.9	11.1	22

(Diet No. 107)

84	71[6]	4.8	11.6	29.3	25.3	60.7	36.4	39.9	2.44	6.16	5.30	12.7	7.64	8.37	10.5	15.6	13.0	16
84	91	5.7	12.1	35.4	30.8	71.1	44.3	48.5	2.16	6.30	5.49	12.7	7.90	8.61	11.1	16.4	12.8	18

(Diet No. 379a)

84	88[7]	5.8	14.9	35.3	30.1	75.2	46.1	47.3	2.56	6.06	5.17	12.9	7.91	8.12	11.3	17.1	10.1	3
105	98	6.2	12.7	38.1	33.2	80.3	48.8	52.1	2.06	6.19	5.39	13.0	7.91	8.46	10.7	15.5	10.6	22

[1] Avg. Final Wt. 284 gms. $\frac{\text{Final Wt.}}{\text{Trunk Lgth.}}$ =12.6; $\frac{\text{Final Wt.}}{\text{Chest Girth}}$ =22.2

[2] " " " 226 " " =10.5; " =19.0

[3] " " " 64 " " =4.2; " =10.0

[4] " " " 75 " " =4.7; " =11.4

[5] Avg. Final Wt. 194 gms. $\frac{\text{Final Wt.}}{\text{Trunk Lgth.}}$ =9.9; $\frac{\text{Final Wt.}}{\text{Chest Girth}}$ =16.4

[6] " " " 57 " " =3.8; " =9.3

[7] " " " 66 " " =4.4; " =11.1

the period of greatest growth. This was found (Sherman and MacLeod 1925) to be due to a lower mineral intake and not, primarily, to a lower intake of the fat-soluble vitamins.

In view of the above differences it was thought of interest to include, in this study of the effect of diet on skeletal growth, a comparison of animals on diet 16 (Diet A) with animals of the same age on diet 13 (Diet B) and to determine whether such moderate but well defined differences as had been found in respect to the general well being of the animals on the two diets would be accompanied by differences in body form and development.

The technique employed in making the various measurements that are discussed in this paper is in brief as follows: Length of body and chest measurements were taken immediately after the animal was chloroformed. All other measurements were made as soon as possible after the death of the animal. With the animal pinned back downward, with nose and tail extended but not under tension, the trunk or body length was determined by measuring with calipers the distance from the tip of the nose to the anus. Chest girth measurements were taken by wrapping a cotton string around the chest at the place of smallest girth and pulling taut but avoiding wrinkling of skin. This string length was then carefully measured with a metric rule. Hip width was taken as the distance between the hip sockets and was determined with calipers resting on the ventral sides of the two cups. The leg bone dimensions were determined with calipers adjusted to fixed points on the sides or ends of each bone. Both right and left leg bones of each animal were measured but no differences in average values were found between them, the measurement of one side simply serving as a check on the other.

The average values resulting from the measurements described above are recorded in Table I. In Table II are given the calculated ratios of the weight of animal to their length of body, chest girth, hip width and length of long bones of legs; also their length of body to girth of chest, hip width, and length of long bones of legs; and length of certain leg bones to their diameter. The weight ratios of Table II are based in each case, with the exceptions noted, on the maximum weight of the animals. The data whose average values appear in Table II were treated statistically when the group contained sufficient numbers to warrant this. The values thus obtained have served in confirming the judgement of the experimenters in the conclusions that have been drawn in this paper. Because of lack of space, the results of the statistical treatment are not herein reported.

The small number of animals examined in many of the younger age groups studied is considered to justify only the conclusion that these points serve as an index of the trend of these particular groups. Variations in the smaller groups however, are somewhat compensated for by their relationship to other groups on the same diet. The number of animals retarded in growth over a period of 8 weeks by a deficiency of vitamin A or vitamin B, and of those over one year of age on diets 13 and 16, is thought to be sufficient to establish these points fairly satisfactorily. It will be noticed that the changes in body form recorded for the different groups are more marked when animals of similar age are compared than when comparison of animals of similar weight is made. Both of these variables should therefore be kept in mind when interpreting the data given in the accompanying tables. Providing sufficient vitamin is added, the vitamin-deficient diets used in this study are adequate for apparently normal growth, although the mineral content is obviously different from that of either diet 13 or 16.

To facilitate discussion of the various groups and to enable comparisons to be more clearly presented, the results of the investigations are considered under the several headings given below.

Animals Restricted in Growth by a Diet Deficient in Vitamin A

Rats from vitamin A experiments recently conducted in this laboratory have furnished excellent material for the study of skeletal growth relationships of animals stunted in growth by a deficiency of vitamin A. Eight animals (negative controls) had been kept on a basal vitamin A-free diet from 28 days of age until their death; 46 animals had been continued on the A-free diet from weaning until the surplus stored vitamin of their bodies was depleted, at which time the basal diet was supplemented by such carefully weighed daily portions of whole milk powder that an average gain in weight of 22–30 grams resulted during an 8 weeks period. This approximates what Sherman and Munsell (1925) have recommended as the rate of growth to be employed in quantitative studies of vitamin A.

The basal vitamin A-free diet referred to (No. 379a) consists of casein 18 per cent, corn starch 67 per cent, yeast 10 per cent, Osborne and Mendel's salt mixture 4 per cent, and sodium chloride 1 per cent.

From a study of the ratios in Table II it is found that the length of of the body, girth of chest and width of hip of the vitamin A-deficient animals increased during the time of restricted feeding to a relatively greater extent than did the weight of such animals during this period.

Ratios of weight to each of these measurements are lower than the corresponding ratios for normal rats of the same age.

Growth in length of the long bones of the legs also continued in animals on the vitamin A-deficient diets, and the ratios of body weight to length of femur, tibia, scapula, humerus, and radius are all found to be lower than the ratios for diet 13 animals of the same age.

Relative to the length of the body, the chest girth of the vitamin A-restricted animals making average gains in weight of 22–30 grams over an 8 weeks period, showed no significant difference from that of normal animals of the same age or weight. In proportion to their body length, these animals have slightly longer leg bones than do normal animals of either the same age or of the same weight. The hip width is relatively narrow when comparison is made with the corresponding measurement of normal animals of the same age, but the difference is not so apparent when comparison is made with normal animals of the same weight. It has been noted that an arching or bowing of the spine is often found in rats stunted by deficient diets (Jackson 1915), (Aron 1914). It is possible that ratios involving the length of the body may be in slight error due to such a condition, but it is probably not an error that would vitiate the measurements in the case of the animals remaining in good health and making the rate of growth described above.

With the possible exception of the humerus, the relative size of the femur, tibia and humerus as shown by the length-to-diameter ratios for each of these long bones is not significantly different from the normal. Although, as has been shown above, the long leg bones of the vitamin A-restricted animal have continued to grow in length relatively faster than the body weight has increased, they have likewise thickened or increased in diameter at relatively the same rate so that quite normal symmetry of these bones results. The humerus shows a tendency to be relatively thicker than the normal in both males and females on the restricted diet, when comparisons are made between animals of the same age, but when comparisons are made between animals of approximately the same weight, the length-to-diameter ratios for the humerus of the animals on the vitamin A-deficient diets are practically the same as the ratio for the normal animal.

Animals Restricted in Growth by a Diet Deficient in Vitamin B

Vitamin B, as the term is here used, is the mixture of the antineuritic substance and vitamin B_2 or G, both being necessary for growth.

For a period of 8 weeks following weaning (28 days of age), thirty-four animals were fed a basal diet free of vitamin B, plus daily weighed portions

of whole milk powder to supply sufficient vitamin B to allow the animals just to maintain their weight during an experimental period of 8 weeks. Forty-four animals were fed from weaning age the basal vitamin B-free diet supplemented with such amounts of whole milk powder that an average gain of 50–55 grams resulted in 8 weeks. The vitamin B-free diet No. 107 consists of casein 18 per cent, butter-fat 8 per cent, cod liver oil 2 per cent, Obsorne and Mendel salt mixture 4 per cent, and corn starch 68 per cent. This diet has been found adequate for normal weight development when sufficient vitamin B is supplied. Previous work in this laboratory has shown that milk is relatively richer in vitamin B_2 or G than in the antineuritic vitamin, hence the latter was the "first limiting factor" in these experiments.

Relative to their body weight, both the animals restricted in growth to barely more than net maintenance, and those making moderate growth during the 8 weeks experimental period, showed longer bodies, greater chest girth, and hip width, and longer leg bones than did normal animals of the same age.

Relative to their body length, the vitamin B-deficient animals were found to have smaller chests than did normal animals of the same age or the same weight. Like the vitamin A-deficient animals, those on the vitamin B-restricted diets showed a tendency to have longer leg bones in proportion to the trunk or body length than did the normal.

The humeri of these animals are longer relative to the diameter than is the case with the other leg bones. It will be seen from Table I that although a definite lengthening of the humerus has taken place during the time the animals were on the vitamin B-deficient diets, the diameter of this bone is practically the same as was found for the normal rats 60 days younger. If we compare the size of the humerus of the animals on the diets low in vitamin B with the size of the same bone from normal animals of the same weight, the difference is even more pronounced than when animals of the same age are compared, the humeri of the vitamin B restricted animals being considerably longer relative to the diameter than is the case with normal animals of the same weight. A condition of bone growth is therefore indicated in these vitamin B-restricted animals that is not common to the other groups studied.

Animals on Diet 16

The animals on diet 16 that were used in this study had been on this diet for from nine to fifteen generations (average twelve); those on diet 13 for from one to twenty-one generations (average nine), with the animals

in each age group averaging at least three generations. They were all taken from the same original stock and all were raised under identical laboratory conditions. In proportion to their weight the young animals from diet 16 have generally longer bodies than do animals from the better diet, No. 13. In the adult animals, however, there is no significant difference in this respect. There appears to be little or no difference in the girth of chest nor in the width of hip, relative to their body weight, of the animals of the same age, or of the animals of the same weight on these two diets.

The young animals on diet 16 generally show longer leg bones in relation to body weight then were found in the case of the diet 13 animals, but for the mature animals, or those over one year of age, the ratios involving these measurements are not significantly different.

In proportion to their body length animals on diet 16 show a tendency when young to have relatively smaller chests and narrower hips than do the young on diet 13 when compared on an age basis, but the difference is insignificant for animals of the same weight. In adult life there appears to be little if any difference in the shape of the body of animals from the two diets.

There is no significant difference from the normal in the length of the tibia, radius, femur, scapula or humerus in relation to the body length of the animals on diet 16 although when compared with the normal there appears to be a slight difference in the size of the tibia, humerus and femur in the very old rats on diet 16. A tendency was found for these bones in animals over one year of age to be slightly thicker or larger in diameter in relation to their length.

It is quite significant that the ratios of measurements of body and bones of animals on the two diets, differing as do diets 13 and 16, should yield results so nearly alike. The small differences found in bone and body form of the rats from the two diets stand in contrast to the larger differences that have previously been found in the life span, reproductivity and resistance to lung infection of the animals on these two diets. Apparently a difference in diet may affect an animal in a serious and subtle way and still cause no marked effect on body form and stature.

Summary

A study has been made of the effects of certain diets on the form and development of albino rats.

Compared with normal animals of the same age, measurements show that in the case of animals receiving a deficient supply of vitamin A there

resulted a lesser retardation of increase in the length of body and of leg, the chest girth, and the width of hips than in the body weight. In proportion to their body length, those animals which received a small amount of vitamin A food, and made gains in weight of 22–30 grams in 8 weeks, show about the same chest girth as normal animals. The leg bones of animals on the vitamin A-deficient diets were found to be relatively longer in proportion to their body length than the normal. With the exception of the humerus, the long leg bones showed no marked difference from the normal in size or shape. The humerus showed a tendency to be thicker in relation to its length, than was found in the case of the bone of normal animals of the same age, but there was practically no difference when comparisons were made between animals of the same weight.

The body form of albino rats stunted by a deficiency of vitamin B is also different from the body form of normal animals of the same age. Compared with normal animals of the same age, the animals retarded in growth because of a deficiency of this vitamin show, as in the case of the vitamin A-deficient animals, that there was a lesser retardation of increase in the length of body and legs, chest girth and width of hips than in body weight. In proportion to the length of the body, these animals have smaller chests and show a tendency to have longer leg bones relative to their body length, than do normal animals of the same age. The humeri, especially, are relatively long and slender compared with the normal.

A study was also made of the development of animals from a stock which for generations had received diet 16, a diet somewhat poorer than the normal, but one that earlier work has shown to be adequate for growth and reproduction through many generations. In the case of the mature animals, and here the larger numbers used in the comparison make the values of more significance, there appears to be no consistent difference in body trunk form of the animals on diets 16 and the better diet, diet 13.

The younger animals on diet 16 generally show longer leg bones relative to body weight than was found in the case of diet 13 animals of the same age, but the differences were negligible for animals of the same weight. The tibias, femurs, and humeri of the very old animals on 16 show a tendency to be slightly thicker or greater in diameter relative to their length than is the case with the very old rats on diet 13.

The small differences that have been found in the bone and body structure of rats on diets 13 and 16 stand in contrast to the larger differences that have previously been found in the life span, reproductivity and resistance to lung infection of the animals on these two diets.

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THE DEVELOPMENT OF THE SUCKLING YOUNG OF MILK FED RATS

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RECENTLY Sure (1), Evans and Burr (2), and Macy, Outhouse, *et al.* (3), have called attention to the fact that the Vitamin B-requirement for lactation is considerably higher than that needed for growth. In nutrition studies, in which the criterion of the efficiency of a given food has been the production and growth of young, these investigators have pointed out that failure in normal development, and ultimate death in many cases, have been due to an insufficient amount of the antineuritic vitamin in the milk secretion. This vitamin deficiency is manifested in the suckling young by posterior paralysis, muscle incoordination, screaming, running fits, and sudden collapse. On postmortem examination, the stomachs were found to be full of curd. Hemorrhages of the bones, especially at the junction of the occipitals and parietals were frequently observed. In the mother there was a marked loss in weight during the lactation period amounting to 20 and 30 per cent of the original weight (4).

That the antineuritic vitamin content of woman's milk, under certain conditions, may be below the physiological requirements of the human infant is well known. In beri-beri the untoward effects of the vitamin deficiency in the nursling are much more marked than in the mother. Even in seemingly adequately nourished women, it appears that the Vitamin B-content of the milk may be lower than has hitherto been appreciated. Macy, Outhouse, *et al.* (5), have observed that from 25 cc. to 30 cc. of human milk were sufficient to furnish the vitamin B-requirement in young rats, whereas 35 cc. were necessary to produce continued growth in larger animals. At this level the animals reproduced, but lactation was unsuccessful due, the authors believe, to a deficiency in the antineuritic vitamin content of the milk. For successful lactation, it appears that from 3 to 5 times as much of this vitamin is needed as for growth. The authors conclude that "the average healthy mother is producing a milk exceedingly low in Vitamin B."

There are few data available relative to the antineuritic vitamin content of cow's milk. Hopkins (6) obtained normal growth in young rats which

were receiving, in addition to a purified ration, 2 cc. of milk as the sole source of vitamin B. Kennedy and Dutcher (7) found 10 cc. per day of either summer or winter milk adequate for the rat; whereas Osborne and Mendel (8), and Johnson (9) were unable to secure comparable growth with less than 16 cc. of cow's milk. Gibson and Concepcion (10), working with polyneuritis in chickens, pigs and dogs, concluded that milk is a poor source of the antineuritic vitamin. Outhouse, Macy, *et al.* (11), obtained excellent growth in young rats during a period of twelve weeks when from 20 to 25 cc. of cow's milk was the only source of the vitamin. Growth then became less satisfactory. The addition of 0.4 gms. of fresh dried yeast brought about an immediate response in growth, whereas the addition of the same amount of autoclaved yeast was without effect. These results indicate that it is the thermolabile fraction which is the limiting factor in preventing the animals from attaining the average adult size on the milk diet. Somewhat similar results were obtained by Sure with dried milk (12). A ration consisting of 50 per cent of dried milk, as the only source of the antineuritic vitamin, produced adequate growth, but failed to promote normal lactation in rats. The addition of a concentrated extract of wheat embryo, made by extracting the embryo with 25 per cent alcohol, or the addition of a dehydrated yeast or yeast concentrate resulted in an immediate response in mammary secretion, demonstrated by the improved well being of the young. These observations led the author to conclude that cow's milk may be so lacking in the antineuritic vitamin as to be inadequate at times for the human infant.

The reported low antineuritic vitamin content of cow's milk suggested that the high mortality of the suckling young of milk-fed rats may be due in part to a too low concentration of this vitamin in the mother rat's milk. If 60 per cent of the vitamin of the food is dissipated in the metabolism and transfer to the milk (13), it seems possible that there may be too little left for proper nourishment of the suckling young. In our experience, however, the young of these milk-fed rats have never manifested the untoward symptoms characteristic of the antineuritic vitamin deficiency.

In order to test this point, we have transferred stock-fed rats to milk diets just previous to, or just following, parturition. These diets have included milk "sterilized" by boiling or pasteurizing, raw milk, and boiled, irradiated milk, as well as several superheated milk preparations, in some of which the destruction of considerable amounts of the antineuritic vitamin has been demonstrated (14). In a certain number of cases

there were added to the milk, substances known to be rich in the antineuritic vitamin. These have included dehydrated yeast, an autolyzed yeast concentrate and an alcoholic (80 per cent) extraction of wheat embryo. In order to rule out the possible stimulating effect of other substances in these, autoclaved autolyzed yeast concentrate, and autoclaved

TABLE I

GROWTH OF SUCKLING YOUNG OF MILK FED RATS

Food*	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
			gms.	gms.	gms.	per cent	
Milk.....	1/31	13	26	132	10.3	....	
Milk.....	2/8	6	20	45	2.4	....	1 died 19th day
Milk.....	3/25	9	28	92	6.2	....	1 died 13th day
Milk+dextrimaltose I 10%..	6/1	7	30	128	9.5	+−0	
Milk.....	6/3	9	26	144	11.4	+6.2	
Irradiated milk.	6/11	7	36	132	9.3	−7.7	Irradiated 20 min. at 2 ft.
Irradiated milk.	6/13	10	32	138	10.3	−4.5	Irradiated 20 min. at 2 ft.
Milk.....	6/12	6	28	106	7.6	−2.2	
Raw milk.....	6/11	6	38	114	8.8	−16.3	Mother killed young 19th day
Milk.....	7/1	8	28	44	2.3	−5.4	Young died on 16th day
Milk.....	11/7	7	24	86	9.4	−3.4	1 died on 19th day
Milk, Past.....	9/15	9	34	72	3.9	+−0	
Milk, Past.....	11/13	10	40	158	11.4	−21.4	
Milk.....	1/31	5	28	128	9.7	+2.1	Second generation
Average....			29.8	107.7	8.03	−4.73	

* All milk was quickly boiled unless otherwise specified.

wheat embryo extract also have been tested. Data pertaining to the development of the suckling young of rats receiving milk to which was added copper sulphate, the ash of wheat embryo, and soy beans respectively, as well as those receiving our stock ration, have been included for comparison. In a few cases the results of the second generation on a given food are reported. To all milk diets small amounts of iron citrate and potassium iodide were added.

In general, the number of young in each litter was reduced to four when the young were four days of age; with a few groups the litters were reduced within twenty-four hours after birth. This seems to have had an

TABLE II

GROWTH OF SUCKLING YOUNG OF RATS FED SUPERHEATED MILKS

Food	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
			gms.	gms.	gms.	per cent	
Desiccated protein milk....	3/2	9	28	148	11.4	+2	
Desiccated protein milk+0.01 gm. $Ca_3(PO_4)_2$ +2 drops of cod liver oil.....	2/2	8	41	116	13.12	–	
Dried Milk No. No. I*.....	3/28	8	48	166	11.4	−10.8	
Dried milk No. I*+.01 gm. $Ca_3(PO_4)_2$+2 drops cod liver oil.....	4/26	9	36	114	7.6	−11.3	
Evap. milk.....	4/24	4	44	174	12.6	−3.9	
Evap. milk.....	5/6	6	30	130	9.9	+3.9	
Evap. milk.....	5/11	9	40	197	15.3	−4.	One killed at 13 days
Evap. milk.....	5/12	9	42	174	12.8	−3.	
Evap. milk+ 10 cc. wheat embryo extract.	2/20	4	28	90	6.03	−14.8	
Evap. milk+0.1 gm.$Ca_3(PO_4)_2$ +2 drops cod liver oil.....	4/27	8	31	196	16.	+−0	
Dried milk No. 2†+16% butter-fat..	11/13	6	40	182	13.8	+−0	
Dried milk No. 2†+16% butter-fat.....	1/31	5	28	162	13.03	+2	Second gen. Boiled milk
Av.....			36	154	11.9	−3.6	

* Milk dried by the spray process.

† Milk dried by the roller process. 16% of butter fat was added to make the composition comparable to Dried milk I.

influence on the results. In a few instances the number raised was less than four, owing to the death of one or two of the group. In these the estimated weight is based on the performance of the two or three surviving. The animals were weighed every three days.

Our criteria of the efficiency of the food have been: 1—the average gain per rat per week, between the fourth and twenty-second day; and 2—the percentage of weight gain or loss in the mother during the lactation period. The results are given in the tables.

TABLE III

GROWTH OF SUCKLING YOUNG OF RATS FED MILK+AUTOLYZED YEAST

Milk *ad lib.* + autolyzed yeast per day	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
0.3	6/9	10	32	180	13.	−8	
0.2	2/17	8	24	76	5.	+−0.	One dead at 9 days. Est. of 4
0.6	6/20	6	32	148	11.3	+7.3	
0.6	11/6	8	32	109	7.5	−4.9	
0.6	11/15	9	37	150	10.9	+−0	Milk Pasteurized
0.6	2/18	8	32	141	10.7	+14.2	One dead at 13 days. Est. of 4
0.6	11/19	9	37	109	7.4	−4.9	
0.6	2/19	10	24	105	8.1	−6.6	Milk Past. Second generation
0.6	2/12	8	25	76	4.9	−6.3	Milk pasteurized
Average			30.5	121.5	8.7	−1.02	

It will be observed that there was considerable variation in the growth of the young of these milk-fed rats, as well as in the gain or loss in weight of the mothers. There seemed to be little if any relation between gain in weight of the young and loss in weight of the mother. The average gains of the young of mothers receiving the superheated milk preparations (Table II) which, with the exception of dried milk 2, have been shown to contain considerably less antineuritic vitamin than equivalent amounts of quickly boiled milk (14), were higher than those of the young of mothers receiving the simple milk diets (Table I), whereas the average loss of

weight among the mothers receiving the superheated milk preparation was slightly less than those of the mothers on the boiled milk. The average gains of the young of these superheated milk-fed rats were only slightly less than the young of the stock-fed animals (Table IV). The mothers of these stock groups, however, either held their own or made a slight gain during the lactation period.

TABLE IV

GROWTH OF SUCKLING YOUNG OF RATS FED MILK + AUTOCLAVED AULOLYZED YEAST*

Milk *ad lib.* + autoclaved autolyzed Yeast. per day	Date	No. born	Weight of litter of 4 at 4 days	Weight of litter of 4 at 22 days	Average gain per rat per week 4 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
0.6	6/7	9	32	130	8.5	+8.3	
0.6	2/19	4	39	150	10.8	+7.	Second generation
0.6	2/22	9	30	172	13.3	−7.	Second generation
0.3	5/21	8	40	170	12.5	−2.	Second generation
0.3	6/28	6	30	128	9.5	+−0	Second generation
0.3	7/12	7	28	130	9.9	−8.	Second generation
0.3	7/12	6	24	152	12.4	+−0	Second generation
Average			32	147	11.	+1.7	

* The autolyzed yeast was dissolved in distilled water and autoclaved at 15 lbs. for 6 hours.

The addition of autolyzed yeast, either autoclaved, or non-autoclaved, was without significant influence on the development of the young, the average in the former group being comparable to that of the superheated milk fed animals, and only slightly less than the stock groups. The young of animals receiving the non-autoclaved, autolyzed yeast concentrate averaged somewhat less than those on the autoclaved, autolyzed yeast. Amongst the mothers receiving the milk and autolyzed yeast, there was considerable variation in the weights during the lactation period. With one exception, in which there was a gain of 14 per cent, these were less marked than those of the superheated or quickly boiled milk groups. The larger amount of autolyzed yeast (0.6 gms. per day), whether autoclaved or non-autoclaved, seemed to protect the mother from the loss in weight

observed among the mothers of the milk and superheated milk groups. The addition of yeast resulted in excellent growth of young in two groups, and fair growth in a third. One mother gained, whereas two lost in weight, one of these more than any other in the series of animals studied.

The young of the animals which received daily 10 cc. of wheat embryo extract in addition to their milk diets gained less than the young whose mothers were receiving the milk only. The additions of copper sulphate, the ash of wheat embryo and of soy beans respectively (Table V) to the

TABLE V

GROWTH OF SUCKLING YOUNG OF RATS FED MILK+MINERALS

Milk *ad lib.*+ per day	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
gms.			gms.	gms.	gms.	per cent	
$CuSO_4$ .0015...	4/28	9	36	168	12.8	−2.	
Wheat embryo ash .071.....	5/1	9	30	158	12.4	−9.5	
Soy bean ash .071.....	5/8	9	38	130	9.	−8.7	
$CuSO_4$ .0015...	7/16	7	32	86	5.2	−18.	Second generation
Average....			34	135.5	9.8	−9.5	

milk rations, resulted in fair growth of young and considerable loss in weight (Av.-9.5 per cent) of the mothers. The combination of wheat embryo ash and wheat embryo extract (Table VI) also resulted in satisfactory growth of young, and slight gains in weight of the mothers.

An attempt was made to determine whether the variation in the results with these milk-fed rats could be related to seasonable changes in milk. The experiments, therefore, were grouped according to the month during which the tests were made. Since there seemed to be no evidence that seasonable variations in the antineuritic vitamin content of the milk is the limiting factor in the development of these suckling young, these tables have not been included.

It is quite possible that there are several factors responsible for the less-than-optimum development in the suckling young of some of our milk-fed rats. In the absence of any one, the others would not be manifest; and although the hypothesis of the antineuritic vitamin deficiency cannot

TABLE VI.

GROWTH OF SUCKLING YOUNG OF RATS FED VARIOUS TYPES OF DIETS

A. Milk+Wheat Embryo Extract*

Milk *ad lib.*+ per day	Date	No. born	Wt. of litliter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or losss 4 to 22 day	Remarks
			gms.	gms.	gms.	per cent	
Wheat embryo extract 10 cc....	2/15	3	34	142	10.5	+3	Second gen. estimate of 4 on wt. of 3
Wheat embryo extract 10 cc....	2/9	7	28	34	1.17	...	Young died at 13 days
Wheat embryo extract 10 cc....	5/9	8	30	62	4.7	...	Transferred to another diet at 15 da.
Wheat embryo extract 10 cc....	9/17	9	32	116	6.2	+2	Second generation
Average....			29	88.5	5.64	+2.5	

B. milk+Autoclaved Wheat Embryo Extract*

Wheat embryo extract autoclaved 10 cc....	3/12	5	28	125	9.4	−2.6	One died at 19 days

C. Milk—Wheat Embryo* and Wheat Embryo Ash

10 cc. wheat embryo extract+ wheat embryo ash .071 gms...	3/5	11	29	166	13.4	+2	
"	3/24	8	28	144	11.3	+−0	
"	7/23	8	28	106	7.5	−5	Second gen. milk past.
"	7/31	4	34	140	10.3	+4.8	Second gen. 2 died on 6th da. milk past. est. 4 on wt. 2
"	7/20	5	34	110	7.4	+−0	Second gen. milk past.
Average....			31	133	9.98	+1.16	

TABLE VI (*Continued*)

D. Milk+Dehydrated Yeast

Milk *ad lib.*+ per day	Date	No. born	Wt. of litter of 4 at 4 days	Wt. of litter of 4 at 22 days	Av. gain per rat per week 4 to 22 days	Mother wt. gain or loss 4 to 22 days	Remarks
Yeast 0.4 gms...	6/24	7	30	132	9.9	−4.5	
Yeast 0.4 gms...	11/3	8	40	142	13.6	−22.7	
Yeast 0.5 gms...	1/11	6	42	168	12.3	+1.6	
Average....			37	147	11.9	−8.5	

E. Milk+Lettuce

10 gms.....	6/3	6	46	164	13.9	−1.9	

F. Stock Ration†

Stock.....	2/12	7	34	158	11.5	+−0	
Stock+lettuce.	4/23	16	20	170	14.3	+−0	
Stock+lettuce.	6/7	11	36	176	13.6	+3.8	
Average....			30	168	13.1	+1.2	

† The stock ration consisted of yellow corn, cooked soy beans to which was added 30 grams of $Ca_3(PO_4)_2$ and 15 grams of NaCl to 4 pounds of dry beans, and milk *ad. lib.*

* An 80% alcoholic extraction of wheat embryo so diluted that 1 cc. contains the extract of one gram of the embryo.

be entirely ruled out, our results offer little evidence in support of it. The excellent growth of the suckling young of the animals fed the superheated milks which have been demonstrated to be lower in the antineuritic vitamin than other forms of heat-treated milks, together with the comparatively slight loss in weight of the mothers during the lactation period, suggests that the variations in these milk-fed animals may be due to the idiosyncracies of the rats and not to any antineuritic vitamin deficiency. It may be difficult in some cases to ingest enough milk to quite meet the caloric needs of the lactating animal, or the flavor and texture of the milk may be distasteful. The fact that our animals have never manifested any of the typical symptoms of the antineuritic deficiency also argues against the hypothesis of the vitamin deficiency.

Contrary to the work of Sure (4), we have found in our milk-fed rats no relation between the gain in weight of the young and loss of weight in the mother. Nor have we found in a series of experiments reported elsewhere (14), that a too low antineuritic vitamin content of the diet of the

mother, manifested in the young by the characteristic symptoms outlined, is consistently accompanied by loss of weight of the mother. Among our milk-fed animals there was excellent development of the young in some cases, and outstanding loss of weight in the mother; in others there was excellent development in the young and no loss in weight of the mother.

Sure (15) has postulated that the antineuritic vitamin content of the cow's milk mixtures used in infant feedings may be below the physiological requirement of the human baby. He has based his conclusions on feeding experiments with rats receiving a ration in which the antineuritic vitamin was furnished by 50 per cent of dried milk. Hoobler (16) also has suggested that a milk mixture consisting of cow's milk and corn syrup (Karo) may contain too little vitamin B for the average baby. Indeed it has been observed by a number of investigators (17) that the additions of concentrated extracts of vitamin B preparations to the milk feedings of the artificially fed infant have resulted in stimulation of growth. Our results with rats do not indicate that the antineuritic vitamin content of milk is so low as to need fortifying in the artificial feeding mixtures of normal infants. A suckling rat may double its weight in six days, whereas an infant may double its weight in four to five months. The young rat therefore grows about twenty-five times as fast as the baby. According to Sure, Evans and Burr, and Macy, Outhouse, *et al.*, from three to five times as much of the antineuritic vitamin is required for lactation as for normal growth. In our experiments one rat suckled four young. It would seem, therefore, that any food which can furnish enough of the antineuritic vitamin for the development of four suckling rats, must contain enough for the normal human infant. It would seem, even, that there would be a considerable margin of safety.

In view of the results with our rats, one is led to wonder if the stimulation of growth in infants following the addition of the vitamin B-containing substances to the artificial feeding mixtures was due to other materials contained therein, or to the fact that the antineuritic vitamin in cow's milk is less available to the human infant. Experiments to determine this are in progress.

Summary

An attempt has been made to determine if the high mortality of the suckling young of milk fed rats is due to a deficiency of the antineuritic vitamin content of the milk. Stock-fed rats, either just previous to or following parturition, were given various types of milk diets, some of which were distinctly lower in the antineuritic vitamin than others.

The influence of the addition of substances known to be rich in the vitamin was tested, as well as the influence of certain inorganic substances.

The results of the investigation indicate that the less-than-optimum growth in some of the suckling young of the milk fed groups is not due to a deficiency of the antineuritic vitamin in the milk secretion, but to the inability of the particular rat to ingest a sufficient amount of milk to meet its caloric requirements, or to a general distaste for the food so that too little is eaten.

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PROTEIN METABOLISM OF CHILDREN ON DIETS EXTREMELY LOW IN CARBOHYDRATES

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The use of diets high in fat and extremely low in carbohydrate in the treatment of idiopathic epilepsy has become increasingly popular in the pediatric clinics of America since its introduction by Wilder (1) and Peterman (2). It was first suggested as a possible substitute for fasting, which had previously been shown by Guelpa and Marie (3) and by Geyelin (4) to cause a cessation of seizures in many severe cases of the disease. The peculiar effectiveness of fasting and of the ketogenic diet was thought by Wilder and Peterman to be due to the anaesthetic action of the ketone bodies formed in the tissues. Other observers believe that it owes its therapeutic value to its effect upon the acid-base equilibrium. The latter believe that it corrects an abnormal tendency toward the spontaneous development of alkalosis in these subjects. While there is some theoretical evidence in favor of each of these more or less plausible explanations, we believe at the present time that the dehydrating effect of such regimens is the primary factor in favoring the cessation of convulsions (5).

No matter what the true mechanism of its action may be, the low-carbohydrate, relatively high-fat diet, together with definite restriction of the total water intake, offers the most effective and most promising type of therapy for the epileptic child. Since the patient must remain on rather limited regimen almost indefinitely, it is extremely important that every precaution be taken to prevent injury to the body from any sort of dietary inadequacy. Not only the total energy value of the diet but its content in protein, vitamins, mineral elements and other necessary constituents must be considered in order that optimum amounts for normal growth and development may be given. The present communication deals chiefly with the phase of the problem relating to the protein requirement.

Practically all of the authors (2,6,7) who have described their practise as regards protein allowance in the strongly ketogenic diets used, have

assumed that one gram of protein per kilogram of body weight is sufficient to meet all requirements. In a carefully conducted study of the protein needs of diabetic children between the ages of 4 and 14 years on relatively high-fat, non-ketogenic diets, Bartlett (8) found that from 0.6 to 1.0 gm. per kilogram of body weight was sufficient to maintain a positive nitrogen balance and to allow normal growth and development, so long as the total caloric requirements were fulfilled. No similar study has been made on children living on the strongly ketogenic diets used in the treatment of epilepsy. In connection with other experimental studies on a series of epileptic children receiving extremely low carbohydrate diets, we have made observations on the nitrogen balance under different conditions. The following is a report of results from six of these subjects.

Plan of Study and Methods Used

The time of each experiment was divided into periods according to variations in the dietary regimens which were being tested as to their efficiency in preventing epileptic seizures. With a few exceptions, the total caloric value of the diet remained unchanged throughout individual experiments. The special influence of the acid-forming and base-forming elements of the diet was determined in two instances. The effect of convulsions on the total amount of nitrogen eliminated was incidentally observed.

The patients, ranging in age from 5 to 15 years, were entirely well throughout the periods of observation except for the convulsive seizures, which they had during certain of the sub-periods. They were kept in bed during periods of fasting and for a portion of each day during the special diet periods, but were allowed to walk about their rooms for mild exercise at other times. Water was allowed *ad libitum* in the experiments reported here, but it may be said in passing that the factor of dehydration, as observed in some of our other studies, has exerted no apparent influence upon the nitrogen balance. The patients were managed in accordance with such requirements as those outlined by Gephart and DuBois (9), so far as this is possible in an ordinary pediatric division, and the diets were carefully calculated from the tables of Atwater and Bryant (10). The total caloric values of the diets were calcualted to be approximately adequate for maintaining body weight under ordinary conditions or for allowing slight gains only. The proteins used were varied, but were chiefly from animal sources. No experiments have been included in which the food given was not all consumed or in which urine specimens were incomplete. All specimens were kept in the refrigerator with toluol

as a preservative. Determinations were always made daily on the 24-hour samples, excepting on Sundays. The latter exception appears to have made little or no difference under the circumstances. Unfortunately, fecal nitrogen could not be determined regularly, but was determined a few times in patients on different levels of protein intake in order that the approximate magnitude of this factor might be appreciated. In these instances the samples were collected in 4-or 5-day periods marked by carmine or charcoal in the usual manner. The total nitrogen of the urine and of the feces was determined by the macro-Kjeldahl method as modified by Folin and Wright (11). The permutit method of Folin and Bell (12) was used for the urinary ammonia. In order that the relationship of ketosis to protein destruction might be ascertained, the urinary excretion of acetone bodies was determined, the colorimetric method of Behre and Benedict (13) being used. For the total titratable acid of the urine the procedure of Henderson and Palmer (14) was employed.

Results

For the sake of brevity and clearness, the data obtained are presented in graphic form on the accompanying charts, which are largely self-explanatory. The body-weight curves in the various charts indicate that the energy values of the rations given were sufficiently high to prevent loss in weight beyond the slight temporary changes due to the dehydrating effect of such diets (15).

When it is desirable to consider the fecal nitrogen, it will be necessary to accept approximate estimations based upon a small number of determinations made on children taking maintenance diets, the constituents of which were similar to those in the present study. The approximate ratios of urine nitrogen to fecal nitrogen for three different levels of protein intake in these cases were as follows: for 4 grams of protein per kilogram, of body weight, 13 to 1; for 2 grams per kilogram, 10 to 1 and for 1 gram per kilogram, 10 to 1. It is obvious that these average ratios are modified by too many factors to be of real assistance in accurate calculations of complete nitrogen balance, but they are of value in indicating the approximate amount of nitrogen lost by this route.

The data recorded in Chart 1 were obtained from a 40-day experiment with an epileptic girl 8 years of age on a carbohydrate-free diet. This period was subdivided into five almost equal periods, in which different levels of protein intake were maintained. The diet remained isocaloric throughout the entire time, a sufficient number of calories in the form of fat being added to replace the protein calories withdrawn with each

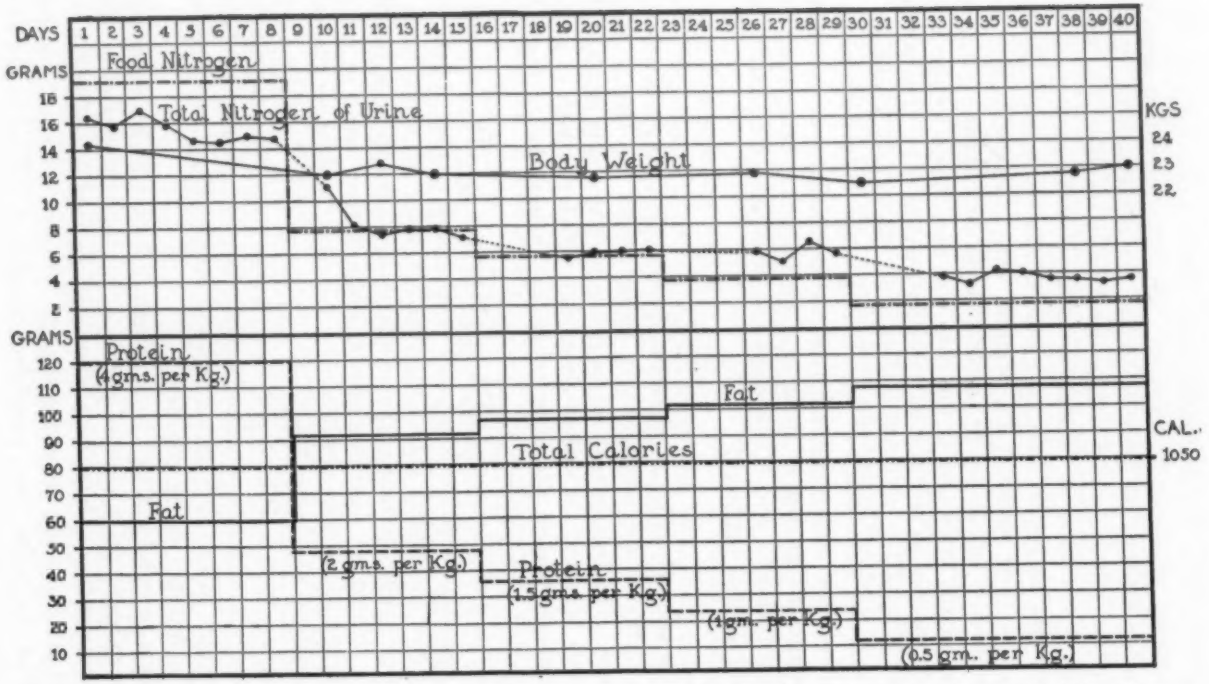

Chart 1. Variations in nitrogen balance on different levels of protein intake. Carbohydrate-free diet. Patient: J. T. epileptic girl, aged 8 years.

change. The initial loss in body weight, amounting to slightly over one kilogram, occurred during the period of very high protein ingestion. There are two characteristics of the diet itself which explain this, namely, its dehydrating effect (15) and the specific dynamic action of the large amount of protein eaten. Assuming the fecal to be one thirteenth as much as the urinary nitrogen, it is apparent from the chart that the patient maintained a positive nitrogen balance in spite of this decrease in weight. During the second period, when the protein intake was 2 grams per kilogram of body weight, the curve representing the quantity of urinary nitrogen practically coincided with that designating the nitrogen intake. An almost identical relationship is seen also between the two in the third period, when 1.5 grams of protein per kilogram were given. There was, therefore, a negative nitrogen balance in these two periods equivalent to the amount of the fecal nitrogen. During the two succeeding periods, when the ratio of ketogenic to ketolytic substances entering into the metabolism was greatly increased, the ratio, food nitrogen: urinary nitrogen, reached a level far below unity. From the data obtained it is estimated that the subject destroyed approximately 14 extra grams of her body protein daily during the last period and 15 grams daily during the preceding period. There were no definite symptoms directly referable to the protein underfeeding during the time of observation.

Chart 2 presents similar data obtained from an experiment on a 13 year old epileptic boy, who had but two mild seizures during the entire period of observation. The diet consisted entirely of protein and fat. Four levels of protein intake, ranging from 4 to 0.67 grams per kilogram of body weight, were tried over periods of 7 days each. There was an initial loss of weight similar to that noted in the previous case, but this was later regained when more fat and less protein were given. It is evident from the chart that the average ratios of food nitrogen to urinary nitrogen for the various periods were not very different from those in the experiment on the younger girl. During the short final period, when 0.67 grams of protein per kilo were given, there was an average daily loss of nitrogen equivalent to 26 grams of body protein.

The comparatively short experiment outlined in Chart 3, is similar to the foregoing, except for the fact that the order of the periods was reversed and the diet contained a small amount of carbohydrate. The subject of the study was a 14 year old boy with moderately severe epilepsy. Inspection of the results reveals the fact that there was a very significant negative nitrogen balance during the initial period when but one gram of protein per kilogram of body weight was taken. During the second

period, however, it is evident that the patient was in nitrogen balance or showed slightly positive balance on 2 grams of protein per kilogram. The food contained 12.8 grams, whereas the urine contained but 11.3 grams daily. There was, therefore, an average of 1.5 grams more nitrogen in the diet than in the urine. If the 10 to 1 ratio of urinary to fecal nitrogen is used to estimate the probable amount of nitrogen lost in the stool,

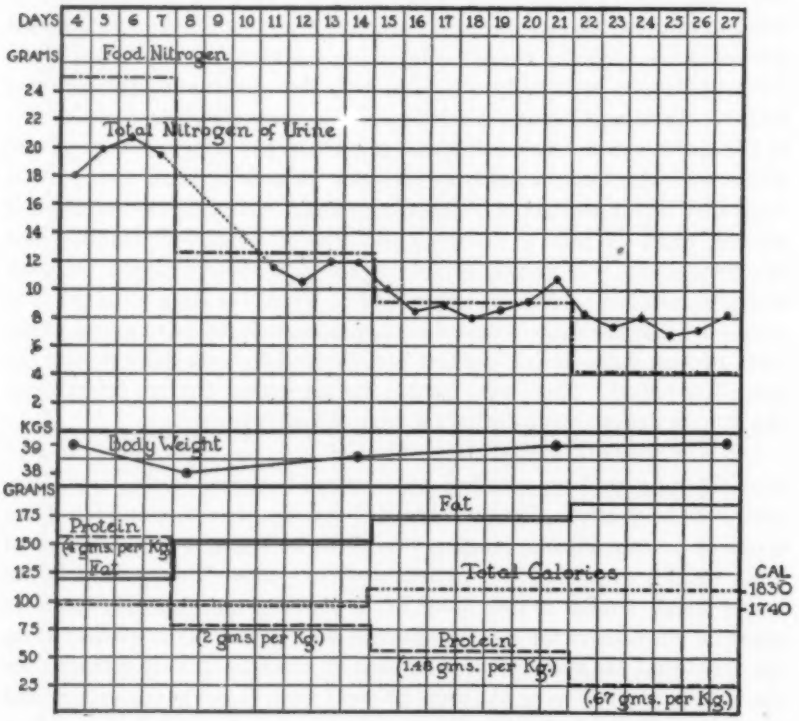

CHART 2. Nitrogen intake and output of J. B., a mildly epileptic boy aged 13 years, on carbohydrate-free diets containing varying amounts of protein.

it is evident that there was an average retention of approximately 0.4 grams daily. For the last period, during which the diet contained 3 grams of protein per kilogram of weight or 19.2 grams of nitrogen daily, the 24-hour output of nitrogen averaged 15.5 grams by way of the kidneys and 1.3 grams (estimated) by way of the intestinal tract. There was thus a retention of nitrogen amounting to approximately 2.4 grams daily. Comparison of these results with those of the previous experiments shows

that even so small an amount of carbohydrate as 15 grams had a very definite protein-sparing effect.

The relationship of the state of ketosis to the nitrogen balance is shown graphically in Chart 4. The data presented were obtained from a study

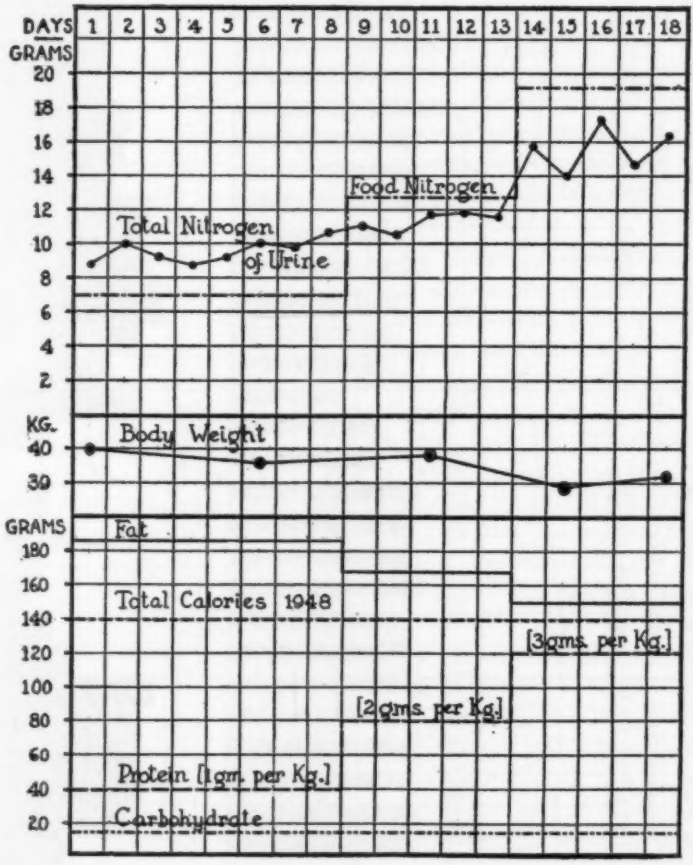

CHART 3. Nitrogen balance study on B. J., an epileptic boy 14 years of age, showing protein-sparing effect of carbohydrate.

made over a period of 34 days on a 15 year old epileptic girl. This differed from the foregoing experiments in that two 3-day fasting periods were included. An interesting fact, which is illustrated here and which we have noted repeatedly is that the degree of ketonuria is diminished tempor-

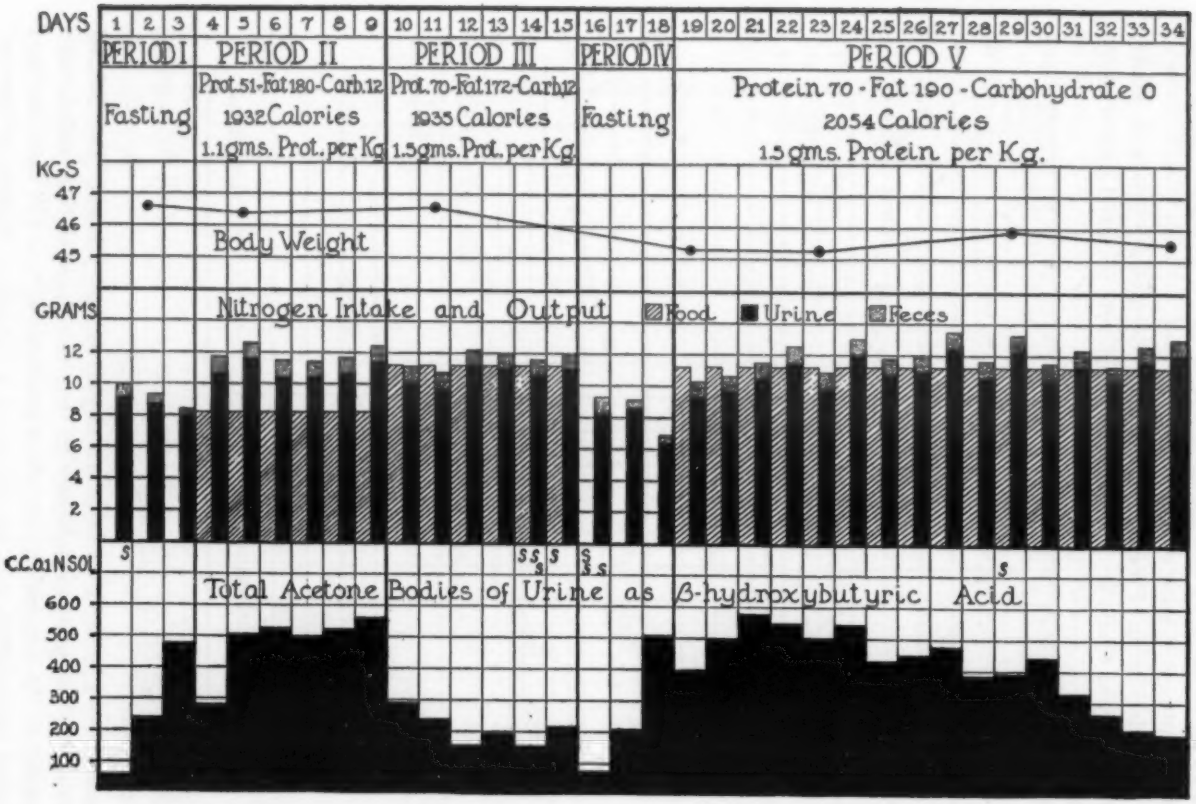

CHART 4. Relationship of fasting and dietary ketosis to protein requirement. J. R., epileptic girl aged 15 years.

arily following the change from fasting to a strongly ketogenic diet or *vice versa*. Weymuller and Schloss (16) also observed the reduction of dietary ketosis by fasting. The explanation, which they suggest for this, is that the proportion of ketolytic material in the mixture metabolized during the early part of the fast is greater than during the preceding period when the large amount of fat was given. At the same time the sharp reduction in metabolism due to the fast tends to spare body fat. Why the intensity of the fasting ketosis is temporarily diminished, as in our experiments, by diets having fatty-acid-to-glucose ratios above 3 to 1 and which later resulted in degrees of ketonuria greater than those occurring during the fast, is not apparent. It is possible that more of the fat from the diet was shunted to the fat depots immediately after the fast periods to replace some of the body fat removed during the fast.

It is evident that the amount of nitrogen eliminated during the periods of fasting diminished in spite of increasing ketosis. Since the patient had previously been on a ketogenic diet with an inadequate protein allowance before each fasting period, this tendency for conservation of body protein may possibly be explained on the assumption that the stores of ' deposit" protein had been partially depleted.

Although the total calories and the amounts of pre-formed carbohydrates remained the same in periods II and III, it is apparent that the average ratio of food nitrogen to total nitrogen excreted for period III (0.97) was very definitely greater than that for period II (0.69), when the degree of ketosis was much more marked. It is obvious that the convulsive seizures which occurred toward the end of period III did not cause a detectable increase in nitrogen elimination.

The diet of period V differed from that of period III in that 18 grams of fat were substituted for the 12 grams of carbohydrate in the latter. The chief purpose of this part of the experiment, as regards the nitrogen metabolism, was to compare the protein-sparing effect of fat to that of carbohydrate under the special conditions present. In spite of the far greater degree of ketosis during period V, the average ratio of food nitrogen to excreta nitrogen for this period (0.94) was barely below that of period III (0.97). Of course, it is obvious from the chart that this average ratio for period V is slightly too high, because there were several days at the beginning of the period during which food nitrogen was evidently being retained to replace nitrogen lost during the preceding fast. The chart, incidentally shows an additional fact of interest as regards non-diabetic dietary ketosis, namely, that there is a tendency for adaptation on the part of the body, such that the degree of ketosis gradually decreases on

a given diet. It is quite apparent, however, that an allowance of 1.5 grams of protein per kilogram of body weight is inadequate in the presence of ketosis, even in older children.

The 23-day experiment represented in Chart 5 was designed primarily to determine the special influence of the non-ketogenic acid-forming and base-forming elements of the ketogenic diet on the seizure tendency in

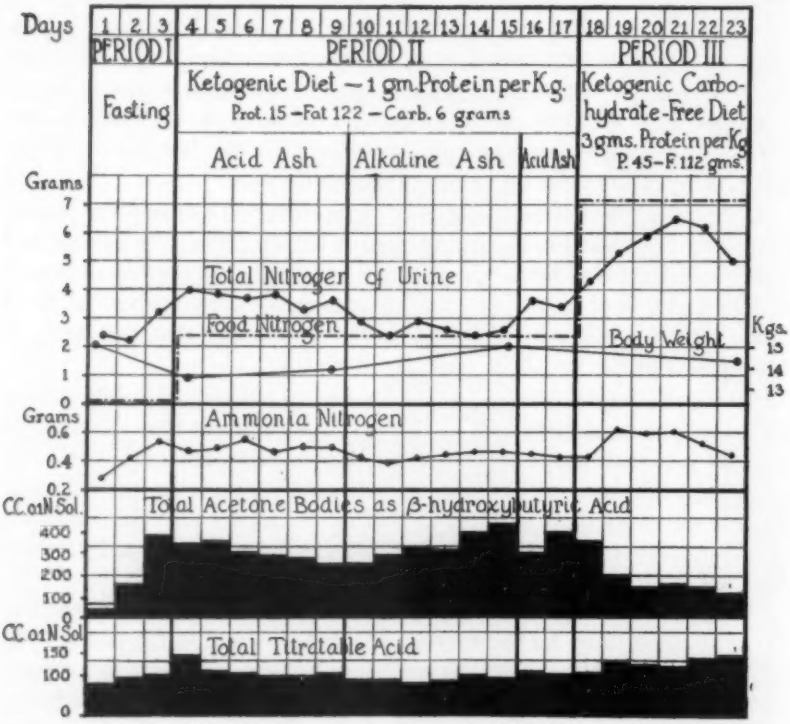

CHART 5. Nitrogen balance during ketosis as influenced by the fixed acid and fixed base content of the diet. S. G. epileptic boy, aged 5 years.

the epileptic patient. Following a three-day preliminary fast, the subject, a 5 year old boy with severe epilepsy, was placed for 14 days on a maintenance ketogenic diet, containing P. 15, F. 122 and C. 6 grams. For the first 6 days the carbohydrate was derived exclusively from the cereal grains, which have an acid ash; while, during the next 6 days, it was given in the form of the leafy vegetables, the ash of which is preponderantly alkaline. Since a considerable amount of bran was included in the diet of

the first 6 days, the amount of indigestible roughage was not very different during the two periods. The base from the base-forming elements in the alkaline-ash was supplemented by the amount of $NaHCO_3$ calculated to be necessary for complete neutralization of the excess of inorganic acid derived from the acid-forming constituents. Following the alkaline-ash period, the diet of the acid-ash period was resumed for two days before the third general period was begun. The latter was characterized by complete lack of carbohydrate in the diet, which, however, remained isocaloric with the preceding.

Following the increase in urinary acetone bodies during the fast, there was a significant rise in the total nitrogen of the urine on the last day. The ammonia nitrogen was increased at the same time but is seen to account for a minor fraction only of the excess total nitrogen. The demand made upon the body protein for ammonia to neutralize the extra organic acids formed under these conditions is far less significant than that for the ketolytic fraction of the protein molecule. This is apparently true for dietary as well as for fasting ketosis. Changes in the total titratable acid of the urine do not correspond directly with those in the total acetone bodies. The only significant change was the gradual rise during the last period of the experiment, when the amount of protein in the diet was trebled.

The most interesting feature of the study is brought out by a comparison of the results during the "acid-ash" and "alkaline-ash" sub-periods of period II. Although the diet for both periods contained the same amounts of protein, fat and carbohydrate, the average ratio, food nitrogen : urinary nitrogen, was 0.65 for the former and 0.92 for the latter. The urinary ammonia was slightly higher during the acid-ash period and the total acetone bodies 15 per cent lower than during the alkaline-ash period. Body weight lost during the fast was rapidly regained during the latter period. A goodly portion of this was probably in the form of water. There were many more convulsive seizures during this period than during the acid-ash periods.

It would appear from this experiment that the added base exerted a protein sparing effect. It has been shown by Rubner (17), and confirmed by others, that indigestible roughage in the diet increases the proportion of nitrogen lost by way of the stools. The difference between the amounts of roughage in the two diets under comparison was too small, however, to explain the 30 per cent decrease in urinary nitrogen during the alkaline-ash period. The small difference in the ammonia nitrogen for the two will account for but a minor fraction of the sparing effect observed. Adolph (18) has shown clearly that the rate of elimination of ingested urea and

ammonium salts in the case of a normal subject is dependent in large measure upon the acid-base equilibrium of the body. He found that these substances were excreted by the kidneys more readily when the balance was toward the acid side and less so in the presence of a reaction in the opposite direction. While the conditions of his experiments and those under discussion were somewhat different, it seems probable that the factor of acid-base equilibrium *per se* might have contributed to the difference in nitrogen excretion in these two periods. However, another possible explanation for the greater amount of urinary nitrogen during the acid-ash period is that body protein was less extensively utilized for its ketolytic fraction during the alkaline-ash period. Haldane (19) has shown that alkalosis induced in a normal man by alkali administration inhibits carbohydrate metabolism to a marked degree and produces ketosis. The increase in the intensity of dietary ketosis together with the diminished nitrogen output in our experiment strongly suggests that the process of breaking down tissue protein for its antiketogenic fraction was held in relative abeyance during the alkaline-ash period because carbohydrate metabolism *per se* was less active.

As seen from the chart, positive nitrogen balance was established during period III, when the diet contained 3 grams of protein per kilogram of body weight. The greater degree of nitrogen retention during the early part of the period probably indicates replacement of the body protein lost during the previous periods. The increase in ammonia nitrogen corresponds with the the increase in the total titratable acidity of the urine resulting from the increased utilization of protein.

That the extra muscular exertion incident to the convulsive seizures had little or no effect on the total nitrogen balance is again evident from the fact that seizures were far more frequent during the alkaline-ash period and during period III than at other times.

In Chart 6 are presented the results of an experiment on a 13 year old epileptic girl, which extended over a period of 64 days. This time was divided into sub-periods, during which various dietary regimens were tested regarding their efficiency in controlling seizures. The total urinary nitrogen, ammonia, acetone bodies and titratable acidity were determined daily throughout the 64 days. The body weight was measured at frequent intervals. The diet remained isocaloric except for two 4-day fasting periods and the last dietary period. In the latter instance it was increased 60 calories daily.

The body weight remained practically uniform when the diet was being given, although there was the expected loss during the fasting periods.

While the degree of ketosis measured for the two fasting periods was practically the same, the nitrogen elimination was very different. In the initial fasting period, which followed a period of comparatively low nitrogen intake, the average daily excretion by way of the kidneys was 5.9 grams; whereas, the average for the second fasting period, which followed an extremely high protein diet, was 11.2 grams.

The data for periods II and III are similar to those of period III of the preceding experiment (see Chart 5). The diets of these two periods were identical, except for the fact that the diet of period III was arranged to have a basic-ash in the manner described in the preceding protocol. The apparent protein-sparing effect of the extra base given during period III is striking when compared with the nitrogen waste of period II. The daily average of the ratio, food nitrogen : urinary nitrogen, for the latter period was 0.66, whereas that for period III was 1.07. The possible reasons for this difference have already been suggested. It is obvious that 0.9 grams per kilogram of body weight is insufficient for complete nitrogen balance in the presence of dietary ketosis. The data of diet period IV show that 1.5 grams protein per kilo of weight is inadequate, when the diet contains no carbohydrate but a preponderance of fat. During period V no carbohydrate was allowed but a sufficiently large proportion of the total calories (over 44 per cent) was given in the form of protein to prevent the development of ketosis. The ketosis existing at the beginning of the period had disappeared entirely by the third day. Although the urinary nitrogen averaged 26 grams per day and the stool nitrogen 2 grams, there was a positive balance of nitrogen, amounting to 6 grams daily. A large portion of the ingested protein was doubtlessly utilized for the antiketogenic portion of its molecule. Another reason for the excessive utilization of protein at this time was the greater demand for energy due to the increased frequency of seizures and to the specific dynamic effect of the protein given. There was probably not only a replacement but an added storage of "deposit" protein during the period, as judged from the excessive output of nitrogen during the first days of the fast that followed.

The diet of period VII was the same as that for period IV and the effects were almost identical, except for the fact that the ketonuria was definitely less marked. It is obvious from a glance at the chart that the average ratio of food nitrogen to urinary nitrogen is slightly less than unity (0.93) as in period IV. In period VIII, there was a substitution of 15 grams of carbohydrate for 15 grams of the protein given in the diet of period VII. Otherwise the two were the same. It is seen that the average ratio, food nitrogen : urinary nitrogen, was lower (0.82) in period VIII, although

DAYS
PERIOD I
Fasting
PERIOD II
Ketogenic Diet with Acid Ash 0.9 gms. Prot. per Kg. Prod. 36-Fat 175-Carb 9
PERIOD III
Ketogenic Diet Alkaline Ash 0.9 gms. P. per Kg. P36-F 175-C 9
PERIOD IV
Ketogenic Carb-Free Kg. P 55-Fat
PERIOD V
Non-Ketogenic, High Protein, Carb-free Diet 1.45 gms. Protein per Kg. Prod. 200 Fat 110 gm
PERIOD VI
Fasting
PERIOD VII
Carbohydrate-Free Diet 1.45 gms. Prot. per Kg. Prod. 55-Fat 170-Carb. 0
PERIOD VIII
Diet of Period VII with 15 gms. C Substituted for 15 gms. Prot. P 40-F 170-C 15
PERIOD IX
Diet of Period VII with Addition of 15 gms. Carb. P 55-F 170-C 15
DIET
KGS.
GRAMS
Body Weight
Nitrogen Intake and Output
Food Nitrogen
Ammonia Nitrogen of Urine
Total Nitrogen of Urine
Total Titratable Acid of Urine
CC. 0.1 N SOL.
Total Acetone Bodies of Urine as Beta-Hydroxybutyric Acid
CC. 0.1 N SOL.

Chart 6. Variations in nitrogen metabolism with different levels of protein intake and different degrees of ketosis. Comparison of nitrogen excretion of fasting periods preceded by different levels of protein ingestion. Effect of fixed acid and fixed base of ketogenic diet upon nitrogen balance. M. P. epileptic girl, aged 13 years.

the daily urinary nitrogen averaged more by 2.2 grams during period VII. The results show that the diet of period IX, which was identical with that of period VII except for the addition of 15 grams of carbohydrate, brought the patient nearer to nitrogen balance, the food-nitrogen to urinary-nitrogen ratio being 1.08. Of course, if the amount of fecal nitrogen is estimated to be 10 per cent as great as that of the urine, it becomes obvious that there was a small negative nitrogen balance for the period.

Discussion

The data presented show very definitely that up to the present time far too little protein has been allowed in the strongly ketogenic diets used in the treatment of epilepsy in children. In the present experiments the attempt was made to determine the approximate amounts required for nitrogen balance under different conditions, no effort having been made to determine the extra amount needed for normal growth of body tissues. It is certain that the ketosis, for which these diets have in the past been given, steps up the protein requirement very greatly. We feel certain that two grams per kilogram of body weight is much nearer the true protein requirement in the very strongly ketogenic regimens than the one gram previously considered adequate.

While we have no definite evidence of harmful effects from our comparatively short-time experiments, it is practically certain that such effects would manifest themselves after many months on such a regimen. We have recently found evidence of a close relationship between the water balance of the body and the occurrence of seizures in epilepsy (5). It is entirely possible that certain patients, who have become refractory to the ketogenic diet, after adhering to it over a considerable period of time, have done so because of a tendency toward abnormal hydration of the tissues secondary to partial protein starvation. The ultimate effect of a diet deficient in protein upon growth and development is too well known to require special comment here.

Whether the protein-sparing effect of added base during dietary ketosis, as demonstrated in experiments 5 and 6 (Charts 5 and 6), might be desirable in some situations is possibly open to question, but its employment in the case of the epileptic subject is rendered impossible by the fact that alkali administration tends to increase the frequency of seizures. The amount of protein required to establish positive nitrogen balance and allow an increase in growth without carbohydrate and with too little fat to permit the development of ketosis, is extremely great, but this can be done. From the observations of Stefansson, regarding the Eskimos, and

of Lieb and Tolstoi (20) on human subjects living exclusively on meat diets, it appears to be certain that no harm is done by diets extremely low in carbohydrate, even when these are used over long periods of time. We have observed that the Eskimo type of diet, even when non-ketogenic, is often effective in preventing epileptic seizures, if the water intake is rather rigidly limited.

Another interesting and probably significant observation, regarding the effect of the low-carbohydrate type of diet upon the general condition of children, is that they appear to remain comparatively free from the common acute infections and complain less of the cold weather during the winter months. It is planned to make some properly controlled studies on these and other similar questions within the near future.

Summary and Conclusions

The extra factors governing the protein requirement of epileptic children on diets high in fat and extremely low in carbohydrate have been studied in six subjects, ranging in age from 5 years to 15 years. It has been demonstrated clearly that the protein allowance, now being used rather widely, of one gram per kilogram of body weight is far too meagre for growing children on strongly ketogenic diets. When as much as one-half to one-third of a gram of carbohydrate per kilogram of weight is being given, one and three-fourths to two grams of protein is probably adequate. More should be given when the carbohydrate intake is lower than this.

The factor chiefly responsible for the excessive demand on the protein under these conditions is the ketosis, which taxes the protein molecule for its ketolytic fraction. When sufficient base occurs in the diet to approximately neutralize the acid from its acid-forming constituents, the rate of nitrogen excretion is definitely decreased. Possible explanations for this are discussed.

The epileptic convulsion *per se* appears to exert very little, if any, direct effect upon the total urinary nitrogen. Possible additional uses for diets low in carbohydrate and high in fat and protein are mentioned.

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We wish to express our gratitude to Miss Clara Husted for making the illustrative charts for this report.

THE VITAMIN B AND THE VITAMIN G REQUIREMENTS OF THE ALBINO MOUSE

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THE fact that vitamin B, old nomenclature (1), consisted of at least two factors, one of which was more heat-stable than the other, was perhaps first clearly demonstrated by testing one vitamin preparation on two species of animals. Thus, Smith and Hendrick (2) reported that an amount of a "vitamin picrate" from yeast that Seidell had found sufficient to prevent the loss in weight of adult pigeons fed solely on polished rice, did not permit the growth of young rats. These rats grew, however, when the "vitamin picrate" was supplemented with a small quantity of autoclaved yeast, a material that Goldberger and his collaborators (3) had demonstrated to be ineffective, even in very large quantities, as a substitute for dried yeast in the diet. Numerous observations in other laboratories have fully confirmed and amplified these early demonstrations of the marked difference in stability towards heat of the two factors that were formerly called vitamin B, not only with the rat as experimental animal but with other species as well. The studies of Hauge and Carrick (4) and of Hogan and Hunter (5) indicated that growing pigeons and chickens may require both the heat-stable and heat-labile factors. It seemed desirable to extend such observations concerning the requirements for these factors, vitamins B and G according to present nomenclature (6), to still another species. We have, accordingly, tested various dietary régimes upon the albino mouse. This species although it is very closely related to the rat, has exhibited certain striking differences in its nutritive requirements; some of them have been described by Beard (7).

In the main, our experiments have indicated that the mouse is no exception to the rule that the heat-stable (G) and the heat-labile (B) vitamins are necessary for normal growth. It has seemed from the investigations reported herein that the mouse requires less of the heat-labile fraction, in absolute quantities, than the rat, a finding that is not without interest since Beard has shown (8) that the growing mouse needs just about the same amount of vitamin B (B+G) as a growing rat. Furthermore, the appearance of certain skin lesions somewhat similar to those described by Goldberger and Lillie (9) as the result of a vitamin G de-

ficient diet, has been obtained more consistently, perhaps, than records in the literature would indicate to have been observed in the rat.

Experiments

In these experiments we have used the special inbred strain of Bagg albino mouse obtained from the Cold Spring Harbor Station for Experimental Evolution through the kindness of Doctor E. C. MacDowell. Some of the young mice were obtained directly from Doctor MacDowell's laboratory and the rest were bred and reared in New Haven from his original stock. Thus, we were assured of the genetic homogeneity of our experimental animals, a matter of considerable importance in mouse work, we believe, since different strains may vary in their growth and behavior on experimental rations. Young mice between three and four weeks of age were taken from their mothers and placed in individual metal cages with wire-screen false bottoms. Because of the susceptibility of mice to sudden changes in temperature, the animal room was maintained between 74° and 80° F. during the entire time the experiments were in progress.

The basal diet consisted of purified casein 31 per cent, purified cornstarch 38 per cent, hydrogenated vegetable oil[1] 24 per cent, and Osborne and Mendel salt mixture[2] 7 per cent. This food, together with drinking water, was available to the mice at all times. In addition the following supplements were fed apart from the rest of the diet. Two drops of cod-liver oil were fed to each mouse daily. One group of mice received daily 200 mgms. of dried yeast[3] per mouse. Another group received 200 mgms. of autoclaved yeast per mouse to furnish the heat-stable factor only. The third group received daily an addition of an alcoholic extract of rice polishings (tiki-tiki) to furnish the heat-labile factor only. The fourth group received daily per mouse 200 mgms. of autoclaved yeast plus two drops, equivalent to two grams of rice polishings, of the undiluted tiki-tiki extract.

The casein was purified according to the directions of Sherman and MacArthur (10) using 60 per cent by weight of alcohol for extraction. This may not have removed the last traces of vitamins B and G from the casein, but it furnished a product of well-defined purity. The cornstarch was also washed with water and extracted with 60 per cent alcohol by weight. The autoclaved yeast preparation was made from the dried yeast by heating in shallow pyrex dishes at 15 pounds gauge pressure for four hours.

[1] The commercial brand "Crisco" was used.

[2] *Jour. Biol. Chem.* XXXVII, 572, (1919).

[3] The product from the Northwestern Yeast Co. was used.

Treatment somewhat similar is known to destroy all of the heat-labile factor, vitamin B, but to leave a considerable portion of the heat-stable factor, vitamin G, unaltered. The vitamin B preparation was made from rice polishings[4] according to the method of Wells (11) for preparing the so-called tiki-tiki extract, using 25 per cent alcohol by weight for the extraction. From two kilos of rice polishings a little over 100 cc. of the sirup were obtained. Evans and Burr (12) have shown that this material is a very potent source of vitamin B but contains very little or none of the G factor.

Our own tests confirm this statement. A group of rats, 21 days old, was placed on a purified diet devoid of vitamin B but containing ample vitamin G as supplied by 200 mgms. of autoclaved yeast daily. The materials used were the same as those employed in the mouse experiments. All of the rats declined in weight and three developed polyneuritic symptoms within four weeks. The food intake decreased during this time but in no case had each animal ceased eating its portion of autoclaved yeast and some of the basal diet when tested in the assay of the tiki-tiki extract. Graded doses of the tiki-tiki extract were given; for this purpose the extract was diluted with 1.5 parts of water so that one drop of the resulting preparation was equivalent to .02 cc. of the original sirup or to .4 grams of the rice polishings. The gain in weight resulting when varying amounts of this source of vitamin B were fed is shown in Table I. Even one drop of the diluted material stopped the decline in weight of vitamin B-

TABLE I

Tiki-tiki extract fed daily in terms of			Average gain per rat in ten days *grams*
Diluted, *drops*	Undiluted, *cc.*	Rice polishings, *grams*	
1	.02	0.4	8
2	.04	0.8	12
4	.08	1.6	39
8	.16	3.2	50

deficient rats and resulted in a slight improvement in a ten-day period. Four drops or .08 cc. gave good growth and eight drops or .16 cc., very good growth. One rat without any autoclaved yeast or other known source of vitamin G showed but slight retardation of loss of weight even when .20 cc. of tiki-tiki extract was fed daily. From these results we may conclude that the tiki-tiki extract used was a potent source of the B factor and contained very little of the G factor, and the rat required at least .08 cc. of this material daily.

[4] This material was secured from the Louisiana Rice Co. of New Orleans.

Results

The results of the mouse feeding experiments are given in the growth curves in charts 1 and 2. With autoclaved yeast alone, all of the animals showed a slight initial gain in weight and then a gradual decline followed by death in 21 days. The mice that received two drops of tiki-tiki extract

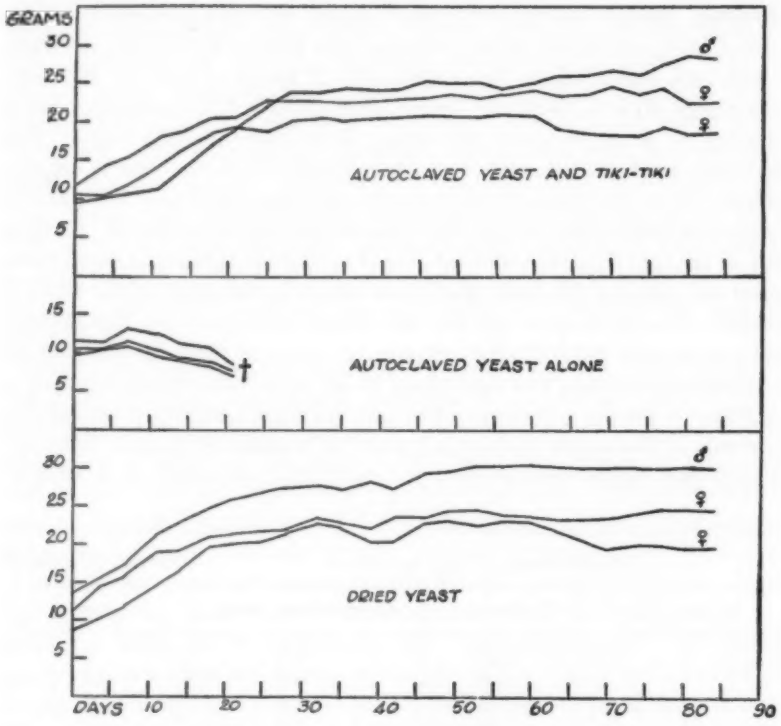

Chart 1. Growth curves of the animals receiving vitamins B (F or B_1) and G(B_2 or P—P) as supplied by autoclaved yeast and tiki-tiki extract, or as dried and untreated yeast. Growth curves of the animals receiving vitamin G only, as supplied by autoclaved yeast.

daily in addition to the autoclaved yeast grew at rates comparable to the group that received untreated yeast. The growth in both cases may be considered normal.

The behavior of the mice that received the tiki-tiki extract only is illustrated in Chart 2. Two drops of the sirup were fed for three weeks during which time the animals did not grow. The animals then showed a

tendency to grow slightly. The tiki-tiki extract was cut down progressively over a period of two weeks to three drops of diluted tiki-tiki, equivalent to .06 cc. of the original extract, every other day. This amount was just sufficient to enable the animals to maintain their weights.

The question of how much of the tiki-tiki extract the mouse required for normal growth, with the diet otherwise adequate, was ascertained by feeding a second group of mice on the basal mouse diet plus daily additions of 200 mgms. of autoclaved yeast and two drops of cod liver oil. No trouble was experienced in getting the animals to eat the autoclaved yeast. Measurements of the food and water intakes showed that these mice ate diminishingly less food and water on the B-deficient diet, during a period of two weeks. At the end of this period none of the mice was eating more than 0.5 grams of food daily. They were then given supplements of graded doses of the tiki-tiki extract. All the animals regained their appetites at once and in three days the average daily food intake was 5.0 grams per mouse. From the results on Chart 3 it may be concluded that .03 to .04 cc. was enough for good growth. Mouse 35 was in poor condition when given the tiki-tiki extract and did not respond as well as the others. Only one mouse, number 33, in this group showed symptoms of polyneuritis, and they disappeared promptly when the vitamin B was given. Throughout these experiments we endeavored to maintain the same conditions as held with the rat assay of tiki-tiki; the materials used in both diets were the same and the experiments ran concurrently. The amount of the tiki-tiki extract required for a growing mouse was only half, or less, as much as a growing rat required. It has been shown (8) that the mouse requires about the same amount of a vitamin B (B+G) preparation as a young rat, or four times as much per unit weight. Although further experiments must be made before any definite conclusions can be drawn about the amounts of each factor needed for normal growth of rats and mice, these few experiments point out the desirability of extending the present quantitative data to a consideration of both the heat-stable and heat-labile factors.

The supplementary action of autoclaved yeast and the tiki-tiki extract is well illustrated by the growth curves of two mice on Chart 2. Mouse 11 received the tiki-tiki extract only for 35 days, during which time it showed no appreciable loss or gain in weight. Then 200 mgms. of autoclaved yeast were added daily for one week. In this time the animal gained in weight and improved markedly in appearence. The tiki-tiki extract was now removed but the autoclaved yeast continued. The growth curve

showed a sharp drop. The animal lost weight rapidly; in 18 days from 18.1 grams to 9.7 grams. Finally, when the tiki-tiki extract was substituted for the autoclaved yeast the mouse improved somewhat and maintained its weight.

Mouse 6 received autoclaved yeast only for 21 days and at the end of this time it was very weak and felt cold. It did not exhibit any of the

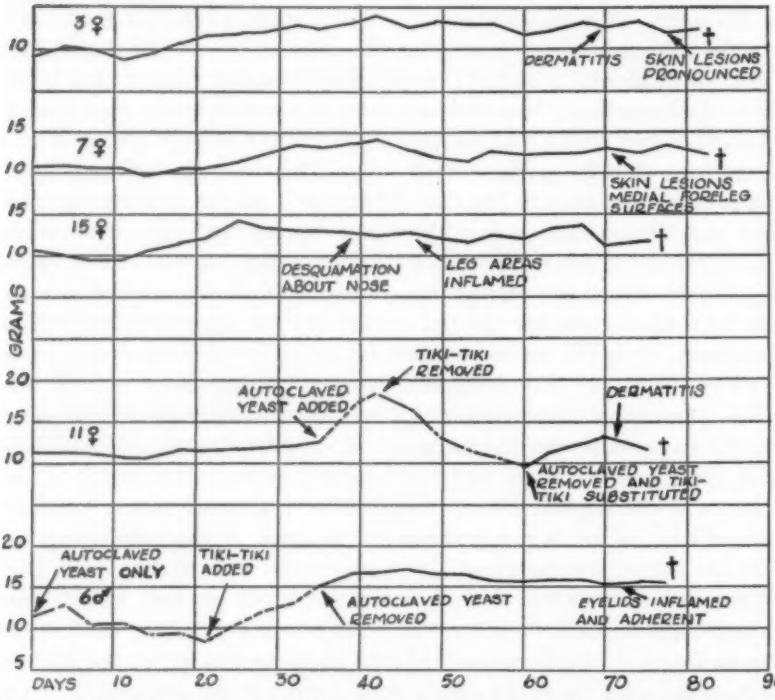

CHART 2. Growth curves of the animals on a vitamin G(B_2 or P—P)-deficient diet, but receiving vitamin B(F or B_1) as supplied by tiki-tiki extract. The lower two curves are of animals that were fed some autoclaved yeast at the times marked on the graph. The arrows indicate also the time when skin disturbances were noticed on each animal.

nervous manifestations that occasional animals show with a similar diet. Two drops of tiki-tiki extract were given by mouth. The animal was alive the next day and the oral administration of the vitamin B preparation was repeated. Thereafter one drop of diluted tiki-tiki extract, equivalent to .02 cc. of the original sirup, was given daily for 15 days. In this time the mouse increased in weight from 8.2 grams to 15.0 grams and improved

markedly in condition. Upon removal of the autoclaved yeast from the diet, growth ceased and the mouse just maintained its weight.

It was observed that the mice receiving the diet deficient in vitamin G, although they did not grow, lived for a considerable time. These mice showed no loss in appetite and consumed their food and water up to the last two or three days of the experiment. This behavior is in agreement

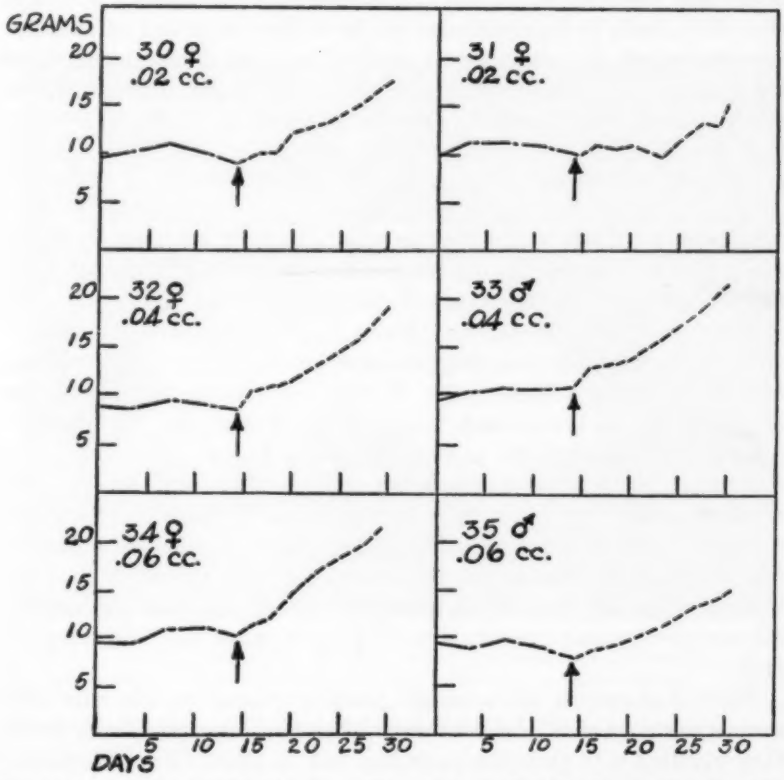

CHART 3. Mouse assay of tiki-tiki extract. The mice received the basal mouse diet plus daily additions of 200 mgms. of autoclaved yeast, and at the point marked by the arrow in each graph, received supplements of graded doses of the tiki-tiki extract as indicated.

with the observations of numerous investigators upon rats. Whether the survival was due to the presence of a small residual amount of the heat-stable factor in the basal diet is difficult to determine. After 39 days on the diet one mouse exhibited signs of skin lesions over the nose. These were first attributed to the animals rubbing against the wire screen of

its cage. However, the skin over the medial surfaces of its hind legs soon became inflamed and other lesions were also observed. The other animals became similarly affected but not until after 70 days on this diet; as is indicated in Chart 2 all the animals on the vitamin G-deficient diet had the skin lesions.

The skin manifestations were very similar to those described by Goldberger and Lillie (9) and Chick and Roscoe (13) with rats on a G-deficient diet. Early in the experiment the fur of these mice had an unkempt appearance but this was no more marked than the derangement of the fur usually seen with almost any deficient diet. Later, the fur became much matted, even though the animals were frequently observed to lick their fur assiduously. The skin beneath assumed a bluish cast; this was particularly noticeable on the abdomen. The first evidence of definite leisons appeared as a slight swelling and inflammation that involved definite areas, usually on the hind legs just over the ankle. In very nearly every case this dermatitis was bilaterally symmetrical. In two of the animals the skin over the surface of the nose was involved, and four had skin lesions under the lower jaw. About four days after the first appearance of inflammation the skin appeared moist from an exudation of serum and the fur began to slough away. A thick yellowish mass of dried serum remained. In the two animals having lesions on the nose, the incrusted mass extended back as far as the eyes, and in one animal involved the eyelids so that these adhered together. Lesions were found on every one of the animals on a G-deficient diet, the most frequent loci being the medial surfaces of both fore and hind legs. Small lesions were sometimes found on the ears, or along the tail, and on the paws. In no case was any healing of the affected regions observed, possibly because the animals did not live very long after the skin disturbances made their initial appearance.

Figure 4 shows the characteristic position assumed by the mice after several weeks on the G-deficient diet. Their hind legs spread wide apart, they squatted with their fore-paws clenched up under them. Frequently the tail was bent back over the body. The exaggerated humped-back and the general appearance of the animals is well illustrated. In no case were any nervous symptoms observed in this group of animals.

When these animals died, immediate autopsy was made, and portions of the skin that showed the lesions were fixed in Zenker's solution and examined histologically. In general the sections from the skin showed the pathology of the later stages detailed by Findlay (14). The epidermis was sloughed away in many places and the exposed derma frequently

showed small ulcerations and infiltrations of polymorphonuclear leucocytes. Although a microscopic study was not made we were not able to observe any congestion or ulceration about the tongue or buccal cavity. The stomach of one animal contained a large hair ball. The intestines of two animals were filled with blood, and the bone marrow of all appeared hemorrhagic. It is interesting to note that the skin lesions on two animals, 6 and 11, developed within a comparatively short time after removal of

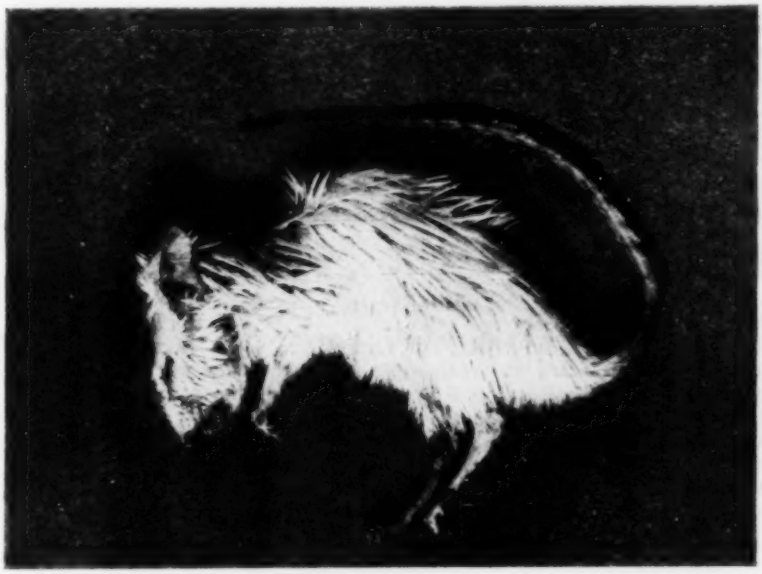

FIG. 4.—Mouse 3, shortly after death as the result of a vitamin G-deficient diet, in a position that is characteristic of that assumed by the animals after several weeks on the diet. Note the appearance of the fur, the arched back, and the dermatitis on the medial aspect of the right hind leg. Lesions are also present on the corresponding surface of the left hind leg, and on the ventral surface of the skin over the neck.

autoclaved yeast from the diet. This fact may be evidence in favor of the opinion of Sherman and Sandels (15) that the skin lesions develop only when there is some of the heat-stable factor in the diet, complete absence of the G factor probably resulting in early death.

The uniformity of behavior of the individual animals on each diet and the general agreement with the results of rat experiments suggests the further use of mice for investigations of this character.

Conclusions

The mouse requires both the heat-stable vitamin G (B_2 or P-P) and the heat-labile vitamin B (F or B_1) in its diet for normal growth and nutrition. In the absence of vitamin B death occurs in about three weeks. In the absence of vitamin G growth does not proceed, but the mice may live for a considerable length of time. This survival may be due to the presence of small amounts of vitamin G in the vitamin B preparation used or to minute residues in the purified basal rations. Skin lesions, similar to those produced in the rat and termed pellagra-like, have been produced in mice on a diet devoid of vitamin G. The mouse required about half as much of the heat-labile vitamin B as the rat.

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AN APPLICATION OF SOME OF THE MORE RECENT METHODS OF ESTIMATING VITAMIN D

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THE line test of McCollum and coworkers, and the methods making use of the chemical analysis of the bone, have been widely used and accepted in the quantitative tests for vitamin D. Recent literature has called attention to certain disadvantages and has suggested new procedures.

One method by Jepchott and Bacharach (1928) makes use of the changing hydrogen ion concentration of the feces, while the other by Poulsson (1928) recommends the use of the X-ray picture in following the rate of decalcification or recalcification. The principles upon which these methods are founded have been described in detail by their respective investigators, and a very complete bibliography covering the entire field has been presented in the recent article by Oser (1928).

A careful study has been made of the application of these methods and certain changes in technique have been suggested. The purpose of this paper is to outline a rapid method of procedure not given in detail in the original articles. A detailed method for the use of the quinhydrone electrode in the *pH* determination has been developed.

Experimental

Albino rats from our own colony have been used as test animals. The colony ration for several years has consisted of a mixture of grains, supplemented with milk powder and minerals. The experimental animals were chosen from stock, 30 days of age, and weighing about 50 grams, divided into lots of four each and housed in a semi-darkened room. They were fed throughout the test period the Steenbock and Black No. 2965 (1925) rachitic ration. When a satisfactory rachitic condition had developed, supplements, diluted in inactive olive oil, were administered to each animal separately from a pipette.

As one of the methods uses the change of *pH* of the feces, considerable attention has been given to the most satisfactory manner of collection of the samples. Gathering from a pan or paper placed beneath a screen

has proved unsatisfactory, due to changes taking place in the atmosphere or by contamination with urine. As a result of many trials, it is now thought to be advisable to secure the feces direct from the animal by gently massaging the lower colon and rectum. With a little practice it is possible to collect samples from a series of animals in a few minutes. Small samples of from .3 to .8 grams have been found satisfactory, an amount that can readily be taken at any time from four animals. This makes possible the collection of the sample and the determination of the hydrogen-ion concentration within a few minutes, thus eliminating the possibilities of error due to bacterial action.

pH Tests

For the determination of the hydrogen-ion concentration Biilmann's (1921) quinhydrone electrode was employed, using as a reference half-cell .01 N HCl and .09N. KCl introduced by Viebel (1923). This makes a convenient and constant reference electrode. Bright platinum electrodes 6 mm. square, fused in glass tubes, were used throughout the experiments. For electrode vessels 100 cc. graduated cylinders were cut at the 25 cc. mark and fitted with glass stirring loops. This permitted the use of small samples. A standard Leeds and Northrup hydrogen ion potentiometer was employed.

The most satisfactory procedure for determining the *pH* of feces received consideration. It was found that dilution and time were factors in the results obtained. From a consideration of data accumulated it is now our custom to use .3 to .8 grams fresh feces macerated in 50 times its weight of distilled water, to which is added 0.2 grams of quinhydrone. The whole is thoroughly stirred for two minutes and the reading taken. This dilution and time are recommended from a consideration of the following facts.

From an inspection of Figure 1, which represents the *pH* dilution diagram, it will be observed that very little change takes place in the *pH* from a dilution of 1 to 30 (1 part feces to 30 parts water) to 1 to 75, therefore a dilution of 1 to 50 would be satisfactory for this work, since no change takes place over a considerable portion of the curve on either side of this point. At these large dilutions a slight error, due either to variation of moisture content of sample, or to an inaccuracy in measurement of the water, would have no appreciable effect on the *pH* of the sample.

A careful study was made in connection with the drift of potential with respect to time. These data are diagrammatically illustrated in

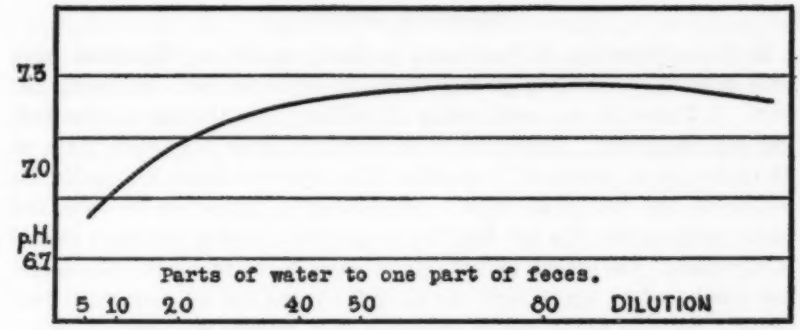

FIG. 1.—Dilution curve showing changes in *p*H due to varying concentrations of the fecal suspension.

Fig. 2. It will be noted that above the *pH* 7.3 there was a tendency for the *pH* to fall, while at low values the tendency was to rise. If, however, the readings are made between 2 and 5 minutes after the addition of the quinhydrone, the error due to the slight drift will be negligible.

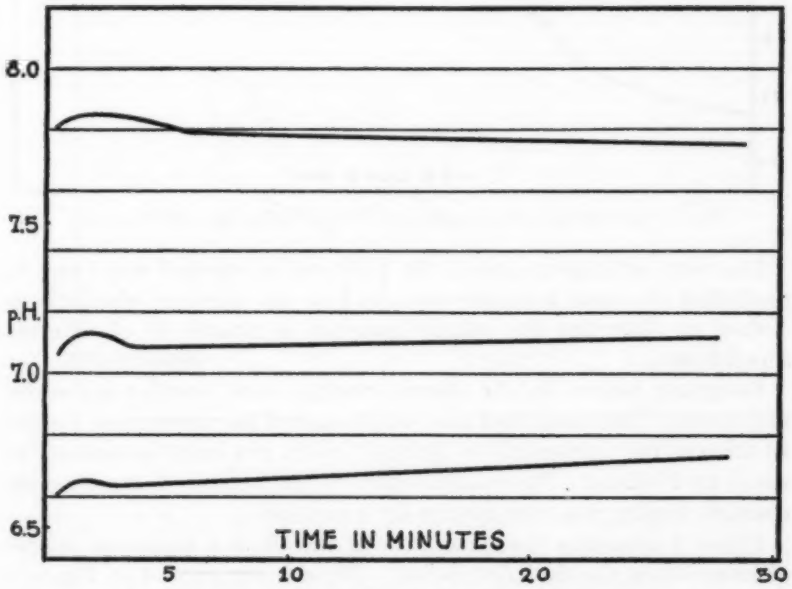

FIG. 2.—Curves showing the drift of *p*H as affected by time at different hydrogen-ion concentrations.

Skiagram Studies

In the application of Poulsson's methods certain modifications have been adopted permitting greater speed of exposures and decreasing the cost. A Fisher X-ray outfit using 10 milliamperes through a four inch gap was employed. Exposures of the left hind knee joint were made at 25 inches for a period of 5 seconds. One operator holds the rat in his left hand, the foot in his right. The animal is turned on its back and firmly held so that the left hind leg is stretched over an ordinary dental X-ray plate. The second person operates the X-ray machine. The plates are numbered to agree with the animal by the use of perforated lead plates. By this procedure an exposure each minute is easily possible.

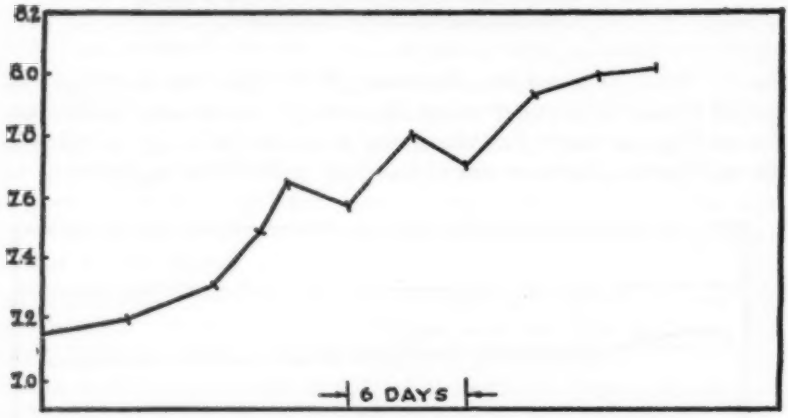

Fig. 3.—Curve showing the increase in *p*H of feces during the depletion period.

The most satisfactory use of the hydrogen-ion method was found in predicting the most favorable time to take the pictures when it was desired to determine the relative amounts of vitamin D of different supplements.

Skiagrams secured by the above technique were found exceptionally satisfactory. The decalcified area which marked the proper time for the addition of the supplement to the basic ration was easily recognized, as noted by Poulsson. The recalcification of the metaphysis marking the complete healing was even more easily recognized.

Figure 3 illustrates the typical rise of the *pH* of a composite sample of feces during the depletion period. There is represented in Figure 4 the response of a number of cages of animals to various treatments following the above mentioned period. Group 1 were controls and the

curve is merely an extension of that of Figure 3. Groups 2 and 3 received cod liver oil, the former an oil of very low potency, the latter a fresh, high grade, oil. Group 4 received an active oil concentrate. The fall of the curves gives evidence for these conclusions.

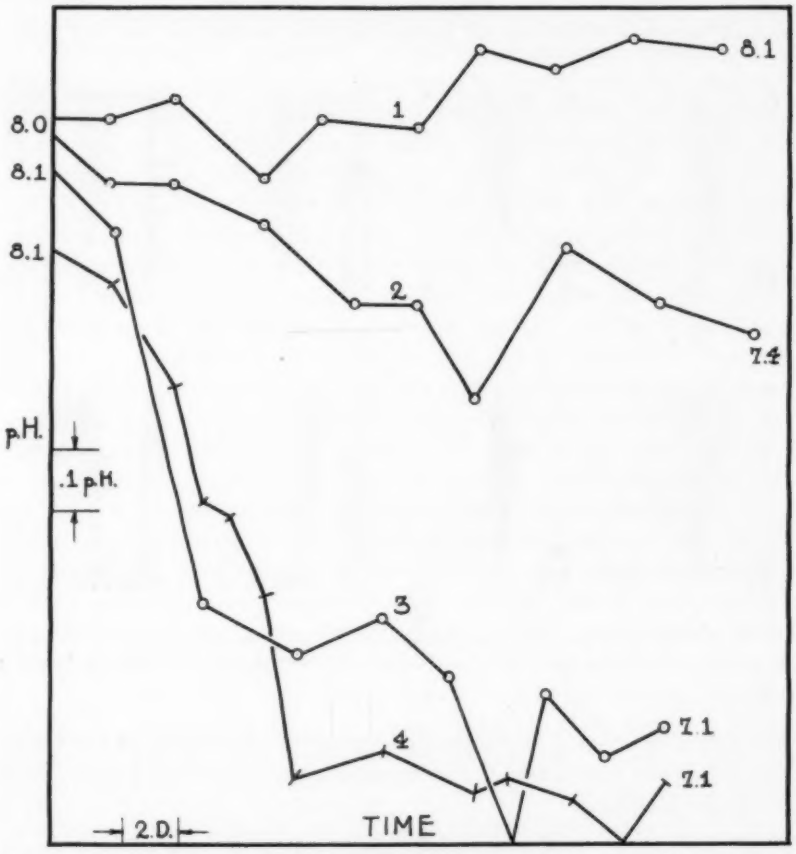

FIG. 4. Curve showing the *p*H of feces of four groups of animals. No. 1 was a control lot. Nos. 2, 3, and 4 received supplements of varying potencies.

Figure 5 shows the skiagrams of the same lots of animals. The upper row of pictures portray the rachitic condition at the end of the depletion period and previous to the addition of the supplements. The lower row of pictures represents the condition of the same animals at the end of test period. Nos. 1, 2, 3 and 4 are skiagrams of the same animals

described as groups 1, 2, 3 and 4 under the previous discussion of *pH* of the feces.

The results are typical of over 60 cages of animals checked in parallel by the two methods.

It is interesting to note that calcification does not take place at the end of the time when the *pH* curves begin to flatten for the first time,

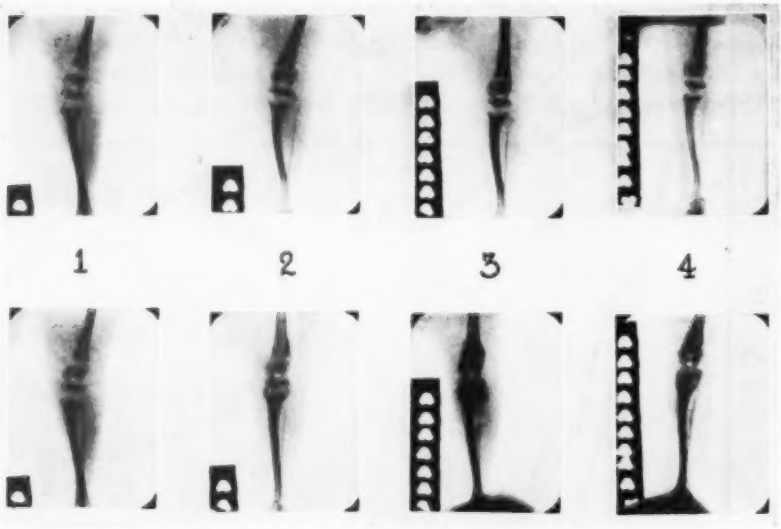

FIG. 5.—The upper row of skiagrams was taken at the end of the depletion period. The lower at the end of the test period. No. 1 is of controls; 2, 3 and 4 received supplements of varying potencies.

but follow it about 2 or 3 days, the change in *pH* being associated with the conditions that produce healing rather than the conditions resulting from the healing.

CONCLUSIONS

The *pH* of the feces increases as the animal bodies become depleted of the vitamin and again drops after vitamin D supplements are added, as indicated by Jephcott. Unfortunately, the daily fluctuations are erratic and smooth curves are never obtained. During the course of this work two groups of investigators, Shohl and Bing (1928) and Oser (1928), have reported this fact and have drawn the conclusion that the

method, therefore, has no practical application. In this conclusion we concur in that we do not feel justified in arriving at complete conclusions as to the relative potencies of two slightly varying supplements. We have found a use for the method, however, that has saved expense and time. In our sixty tests it has been found that if a curve be drawn through the mean of the points, the relative slope of the curve is usually comparable to the findings of the skiagrams. It must be emphasized, however, that this holds true only when the same basic ration is used and that the supplements must be of similar nature, such as cod liver oil or its concentrates, and the method does not hold if supplements are added that change the basic ration materially.

The method finds its usefulness in combination with the Poulsson method. When the *pH* curve ceases to rise and flattens out, one recognizes the proper time for the first X-ray exposures, and again, after the addition of the supplements the drop of the curve and finally the reflattening, indicate the time for the second exposures. Our practice is to wait two days after the break of the curve of the animal receiving the most potent supplement, and then take the pictures of the entire series to obtain the most comparable results. An examination of the curves indicates that the drop of the *pH* does not parallel the recalcification, but precedes it by three or four days. At least, during the period of greatest drop of *pH*, no evident changes are shown in the skiagrams. The combination of the two methods makes it possible to keep a daily record of changes of a large series of animals throughout the entire period with a comparatively small amount of labor and expense, and the results are more satisfactory than the conclusion based only upon a single examination at the end of the period.

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THE INFLUENCE OF A LOW-FAT DIET UPON FAT METABOLISM DURING LACTATION

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While there are many experimental data indicating that milk fat has its origin in part at least in dietary fat, it is also recognized as a result of the experiments by Jordan and coworkers (1, 2) that with a low-fat diet carbohydrate can be utilized as a source of the fat in the milk. These experiments raised the question, which has been little studied and never satisfactorily answered, as to whether the substitution of carbohydrate for fat as a source of milk fat has any influence on the quantity or quality of the secreted product. The present investigation was undertaken to study this question and to obtain data which might result in a better understanding of the physiology of fat metabolism during lactation.

There are recorded in the literature a large number of experiments dealing with the influence of food fat upon the secretion of milk fat. Insofar as the influence on the quantity of secretion is concerned, the results as a whole are contradictory and inconclusive. A review of the earlier work is given by Basch (3) and by Porcher (4). Many of the experimental data reported with low-fat diets are inconclusive either because of the short duration of the experiment, or because of the presence of other variables such as differences in protein or energy intakes in the rations compared.

In the studies with cows by Jordan and coworkers (1, 2) in which the ration was made low in fat by benzine extraction, it was demonstrated, by determining the amount of fat digested and the nitrogen balance, and by other data, that the fat secreted could not have come from fat in the food or depots or from protein, but must have been made in part at least from carbohydrate. These experiments have been cited by some reviewers as evidence that the fat content of the diet is of no importance for lactation. Such a conclusion is not justified by the data. In fact, in the first report the data show that the substitution of the extracted feed resulted in a lower yield of milk and fat. However, this result is inconclusive because in changing from the unextracted to the extracted ration the intake of digestible nutrients was lowered. The writers themselves did not draw any conclusions as to whether or not a low-fat diet is unfavorable to milk and fat secretion. The importance of their work lies in the clear demonstration that milk fat can be made from carbohydrate.

Morgen, Beger and Fingerling (5, 6, 7) have reported a very large number of studies with sheep and goats from which they conclude that while carbohydrate can be utilized to manufacture milk fat, fat itself is a more suitable material and that within certain limits the percentage of fat in the milk is influenced by its content in the feed. Specifically, it was found that rations containing approximately 1 gram of fat per kilo live weight resulted in a larger secretion of fat and, to a lesser degree, of milk, than rations containing 0.5 of a gram of fat per kilo live weight. Though fairly consistent the differences obtained were small in most cases. The significance of many of the data is questionable because the rations compared differed as regards their ingredients and also as regards the quality of their protein and other factors. Since the secretion of milk and fat by different animals, which were similar in weight, varied as much as 500 per cent, the use of live weight as a basis of fat intake also tends to obscure the significance of the results.

In some additional studies by Fingerling (8) with goats, carried out similarly to those just described, the same conclusions are drawn. In these experiments the intakes of digestible protein and of starch value were kept constant, but again one feed was substituted for another in shifting the fat intake, and live weight was used as the basis for the latter.

The data reported in the papers just reviewed resulted in the inauguration of a cooperative investigation of the question with cows, carried out in ten agricultural institutions in Germany. This investigation has been reported by Kellner and coworkers (9). The rations compared were equal in "starch value," but one was richer in carbohydrate and the other richer in fat. The former contained approximately 0.5 of kilo of digestible fat per 1000 kilos live weight and the latter approximately 1 kilo per 1000 kilos live weight. The higher fat level was provided by substituting rice meal for barley meal and starch, which were used as concentrates in the low-fat ration. The data obtained failed to show any higher yield of milk or fat as a result of the high-fat ration. An analysis of the rather variable data shows that even the low-fat rations contained nearly as much fat as was secreted in the milk. In fact some of them contained more. Thus, the experiments can not be considered a rigid test of the effect of a low-fat ration.

Experimental Procedure

Four cows were alternately fed a typical dairy ration consisting of alfalfa hay, beet pulp and a grain mixture, and the same ration from which

most of the fat had been removed by extracting the grain mixture with benzine, the extracted fat being replaced by an isodynamically equivalent amount of starch. During a period of 30 days two cows were fed the normal-fat ration and two were fed the low-fat ration. The rations were then interchanged for another period of 30 days, and then shifted back to the original basis for a final period of 24 days. This system of feeding is shown in the charts in which the results are presented.

The rations used were chosen with the object of providing optimum nutrition, aside from the possible effect of the removal of the fat. The hay used was second-cutting alfalfa of excellent quality. The beet pulp was used as a succulent feed by soaking it for several hours prior to feeding. The grain mixture was made up according to the following formula:

150 pounds cottonseed meal, 43% protein
150 pounds linseed meal
450 pounds hominy feed
250 pounds wheat bran

A sufficient amount of the above was mixed prior to the start of the experiment and divided into two portions. One portion was set aside as the normal grain mixture. The other was extracted by the benzine process,[1] and the fat removed, as shown by analysis before and after extraction, was replaced by an isodynamically equivalent amount of starch.

The analyses of the various feeds are shown in Table I. The figures for digestible nutrients are calculated values based on the average coefficients of digestibility published by Henry and Morrison (10).

TABLE I
ANALYSES OF FEEDS

Feed	Crude protein %	Carbohydrates		Fat %	Dig. crude protein %	T.D.N. %
		Fiber %	N.F.E. %			
Normal grain mixture	19.53	7.34	53.96	5.78	14.70	73.06
Low-fat grain mixture	19.20	7.07	60.08	0.66	14.42	68.65
Beet pulp	9.24	20.32	59.12	0.64	4.80	70.77
Alfalfa hay	15.52	28.37	37.10	2.25	11.81	53.77

The cows used were mature Holsteins, at least 2 months along in their lactation period at the beginning of the experiment. All were bred shortly

[1] The extraction was carried out by Oil Processes, Inc., Harrison, New Jersey.

before the start of the experiment with the exception of one, number 4, which was bred 10 days thereafter. Their production for 30 days before going on experiment is shown in Chart 1. Their ration during this period had consisted of mixed hay of rather poor quality, corn silage and a 20-per-cent-protein grain mixture. The ration for each cow was adequate for her weight and production, according to the Morrison standard.

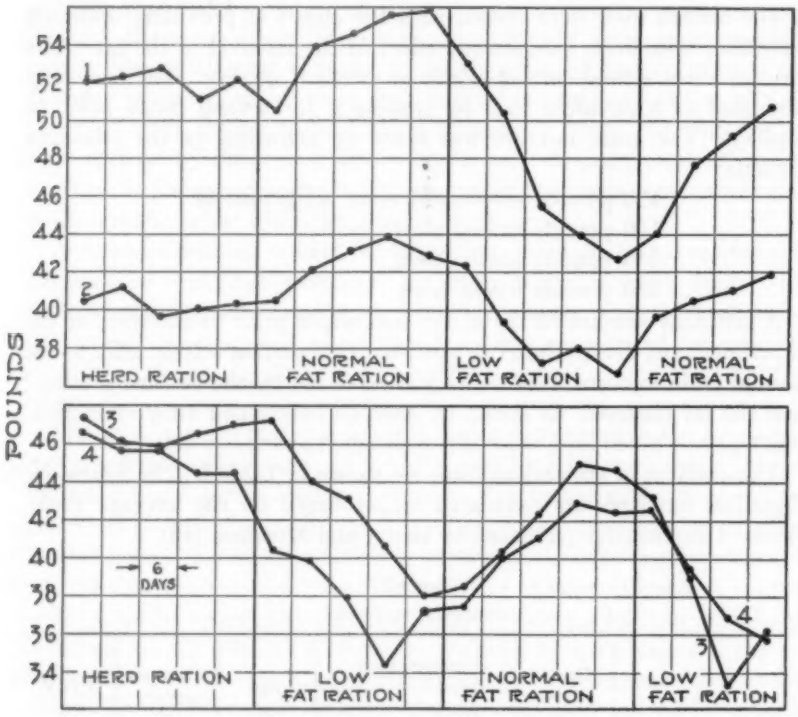

CHART 1. Average daily milk yield.

During the experiment the ration fed each cow was recalculated every 6 days. It was based upon her production of milk and fat during the previous 6 days and upon her average body weight for 3 consecutive days during this period. The normal-fat grain was fed at the rate of 1 pound for every 3 pounds of milk produced. Since the low-fat grain contained approximately 6 per cent less total digestible nutrients as shown in Table I, due to the substitution of starch for fat, it was fed at the rate of 1.06

pounds for every 3 pounds of milk. Each cow received approximately 1 pound of hay and 0.6 pounds of beet pulp per 100 pounds liveweight, but the ratios were varied as necessary to provide each cow with total digestible nutrients in accordance with the Morrison standard and with digestible protein approximately 20 per cent in excess of this standard. In deciding to use alfalfa hay with the 20-per-cent grain mixture it was, of course, recognized that the plane of protein intake would be unnecessarily high. There seemed to be no disadvantage in this and the use of the best roughage appeared distinctly advantageous from the standpoint of providing a ration optimum in all respects other than its fat content.

The cows were milked at 5 A.M., 1 P.M., and 7 P.M. The grain and beet pulp were fed before each milking and the hay was fed at 9 A.M. and 4 P.M. The beet pulp was soaked several hours and the grain was mixed with it just before feeding. Approximately 1.5 ounces of salt were fed to each cow daily. All feed was accurately weighed and any refused feed was accounted for. With the exception of one short period in the case of two of the cows, as will be mentioned in detail later, the rations were completely consumed.

The milk was sampled at each milking, taking 1 cc. for each pound produced. These subsamples were composited over a period of 6 days, analyzed for fat by the Roese-Gottlieb method and the iodine number of the fat determined by the Hanus method.

Blood samples were taken approximately every 10 days throughout the experiment. The samples were taken from the jugular vein, always at the same hour, 11 A.M. The blood was analyzed for fatty acids by the oxidation method of Bloor (11) and for cholesterol by Bloor's colorimetric method (12).

The cows were weighed on 3 consecutive days during each period of 6 days and the average taken as the weight for the period.

Results

The daily milk yields were averaged for periods of 6 days and these average values are plotted in Chart 1. To show the previous production of the animals, their yields are plotted for 5 six-day periods prior to the start of the experiment. In studying this chart and succeeding ones it should be noted that each point is plotted in the middle of the six-day period for which it represents an average value. The change to the experimental rations was made abruptly. It is noted in the chart that the yields of cows 1 and 2 rose when the animals were changed to the normal-fat ration, and that when they were changed to the low-fat ration their

yields dropped sharply and continued to drop throughout this period. When the cows were put back on the normal fat ration, it is seen that their yields started up again and continued to rise until the end of the experiment. The curves for cows, 3 and 4 show that these animals responded to the changes in the fat content of the ration in an entirely similar way. When changed from the herd ration to the low-fat ration their yields dropped sharply, rose nearly to their original levels on the normal and dropped again sharply during the final low-fat period. The fourth value plotted for cow 4 during the first low-fat period is abnormally low because during a portion of the 6 days represented by this value the animal was suffering from a sore on her jaw and thus failed to eat all her food. The disappearance of the trouble is reflected in the higher yield shown for the final 6 days of the period. Similarly the sharp dip and subsequent rise in the curve for cow 3, at the end of the final low-fat period, was caused by the animal's going off-feed. During the third period of 6 days this animal consumed only about half her ration and thus her yield fell off markedly. In the succeeding period she ate her entire ration and thus her yield came back. Aside from these two irregularities in the curves for cows 3 and 4, which are entirely explainable on the above basis, the curves are remarkably uniform in showing that the change in the fat content of the ration had a marked effect upon the yield of milk.

To make more certain regarding this conclusion, it is desirable to know whether the intakes of total digestible nutrients during the different periods were adequate and similar in accordance with production. The data in Table II furnish an answer to this question. The figures for the total digestible nutrients required by the Morrison standard were computed on the basis of the cow's average weight and her yield of milk and fat during the period represented by each ration. The figures for the actual intake were obtained from the records of food consumption and the data as to percentage composition of each feed, using the average digestion coefficients as published by Henry and Morrison (10) to compute the total digestible nutrients. These data for the theoretical requirements and for the actual intakes could have been computed for each six-day period, but this did not seem necessary for the purpose. For the data as a whole the correspondence between the required intake and the actual intake is remarkably close.

Assuming the substantial accuracy of the feeding standard and the applicability of the average digestion coefficients, it is clear that the drops in production which occurred on the low-fat ration and the increases that occurred on the normal were not due to changes in total food intake and

they must have been due to the changes in the fat intake. The only low-fat period during which the actual intake was significantly less than the theoretical requirement, was the last one for cow 3. This was due to her failure to consume the ration allotted her during one period of 6 days, as previously explained.

TABLE II

DAILY INTAKES OF TOTAL DIGESTIBLE NUTRIENTS COMPARED TO REQUIREMENT BY MORRISON STANDARD, AND DAILY INTAKES OF DIGESTIBLE FAT.

Cow	Ration	Total Digestible Nutrients		Digestible Fat lbs.
		Requirement by Morrison Standard lbs.	Actual intake lbs.	lbs.
1	normal	23.6	23.4	1.02
	low-fat	22.4	22.3	0.23
	normal	23.2	23.3	0.94
2	normal	23.3	23.2	0.87
	low-fat	22.4	22.6	0.24
	normal	23.7	24.0	0.85
3	low-fat	23.6	23.9	0.25
	normal	24.9	24.0	0.87
	low-fat	23.2	22.5	0.24
4	low-fat	20.0	20.0	0.21
	normal	21.0	20.9	0.81
	low-fat	20.4	20.6	0.22

In drawing the conclusion that the production dropped as a result of the lower fat intake and despite an adequate intake of total food, the possibility is recognized that it may have been due to the removal of some other dietary essential besides fat. This possibility seems remote. While some fat-soluble vitamins were doubtless removed, previous studies indicate that this would affect the content of the milk in these vitamins rather than the yield. It is also possible that the removal of the fat lowered the digestibility of the entire ration and that the intake of total digestible nutrients was actually inadequate, although shown to be adequate on the basis of the calculation using average coefficients. While this may deserve study by including digestibility determinations in a repetition of the experiment, the possibility that the digestibility was sufficiently lowered to cause the large drop in yield recorded seems remote.

Table II also contains the figures for the average daily intakes of digestible fat for the different periods. Average digestion coefficients were used in computing these values. It is seen that the intake of digestible fat was reduced by approximately 70 to 75 per cent when the low-fat ration was substituted for the normal.

The curves in Chart 2 show that the change from one ration to another was without effect on the weights of the animals.

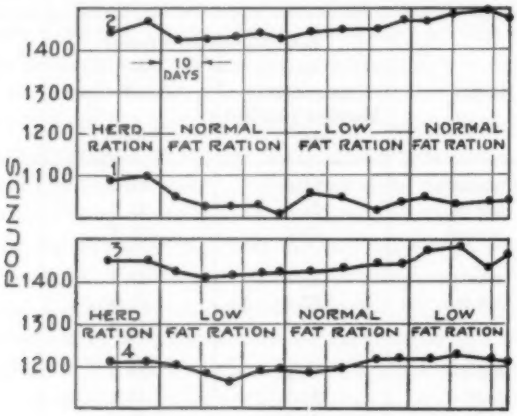

CHART 2. Weights of cows.

The data obtained by analyzing the six-day composite samples of milk for fat are shown in Chart 3. The data show a high degree of variability. It has long been recognized that fat is the most variable constituent of milk. Clearly there is no indication in Chart 3 to support the conclusion of Morgen and coworkers (5, 6, 7) and of Fingerling (8) that a low-fat ration lowers the percentage of milk fat. On the contrary, if the curves show anything at all, it is a tendency for the percentage to rise on the low-fat ration and to fall on the normal. However, any such tendency should not be considered a direct effect of the changes in the fat intake. Rather it may be considered a result of the changes in yield. There are many data showing that as the yield of milk falls, the percentage of fat tends to rise. Particularly this is noted toward the end of the lactation and at other times when the yield suddenly drops for any reason. The high value obtained for cow 3 in the next to the last six-day period is an illustration of this since, as is shown in Chart 1, this is the period during which her milk yield took a very sudden drop as a result of failure to eat.

Many of the conflicting data in the literature are due to the failure to recognize the normal variability in the fat content of milk and the frequent inverse relationship of this percentage to the yield.

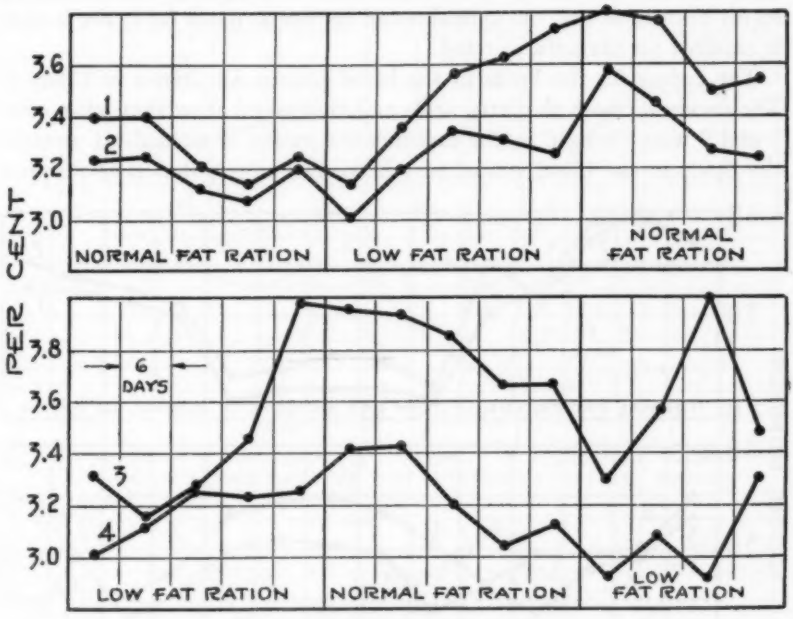

CHART 3. Percentage of fat in milk.

The curves for the daily yield of fat are shown in Chart 4. Although these curves are irregular, as is to be expected in view of the variability of the percentage of fat, it is apparent that the yield of fat tended to be lower on the low-fat ration. It is clear from the data presented in charts 1 and 3, however, that this lower fat yield is due to a lower yield of milk as a whole, and not to a lower percentage of fat. While at first thought this may seem difficult to understand, it should be remembered that, though the fat percentage may vary within certain limits under the influence of a variety of factors, the mammary gland tends to secrete a product of constant composition. On this basis a lack of suitable raw material for the manufacture of a given nutrient will tend to reduce the secretion as a whole.

A comparison of the data in Table 2 for the intake of digestible fat with the data presented in Chart 4 shows that the normal fat ration contained

approximately 60 per cent as much digestible fat as was secreted in the milk, whereas the low-fat ration contained only about 18 per cent as much. It is a question worthy of study whether or not the milk secretion would be increased beyond that obtained on the normal ration by increasing its fat content so that the animal would receive as much fat in her feed as is required for the milk secreted.

The curves for the lipids in the blood plasma are shown in Chart 5. The curves for both the fatty acids and cholesterol show that when cows 1 and 2 were changed to the experimental ration of normal-fat content the lipids in the blood tended to remain unchanged, that they dropped

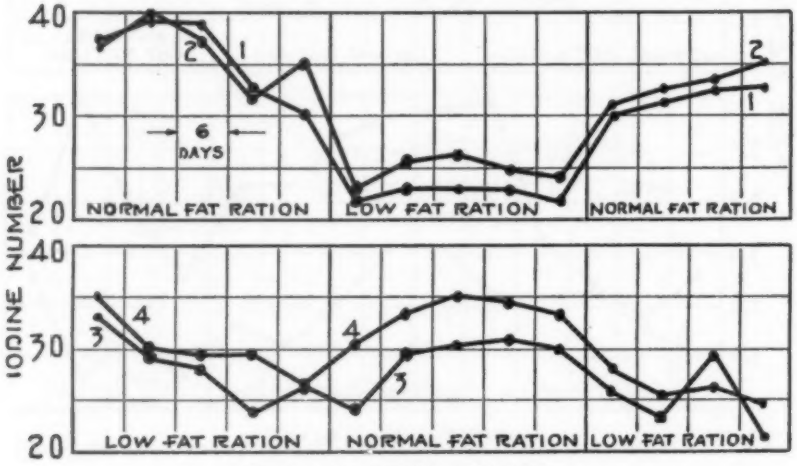

CHART 4. Average daily yield of fat.

sharply when the low-fat ration was substituted, and continued to drop until the normal ration was again fed. They then gradually rose to their previous normal values. The values for cows 3 and 4 are seen to drop during the low-fat period, rise during the normal, drop again during the second low-fat period, and return to normal when placed again on the herd ration. The latter contained somewhat more fat then the normal-fat experimental ration.

It is noted that there is a close parallelism between the curves for fatty acids and cholesterol similar to that shown by Terroine (13) for dogs. There is also a striking parallelism between the trends of the curves in Chart 5 and those for milk yield shown in Chart 1. It should be noted that the drop in blood lipids on the low-fat ration and the subsequent rise when the

ration higher in fat was substituted is not due solely to alimentary fat. The full influence of the latter should be shown at the first observation in the period in question, since this observation was not made until 7 or 8 days after the change, whereas it is seen that the drop or rise continued

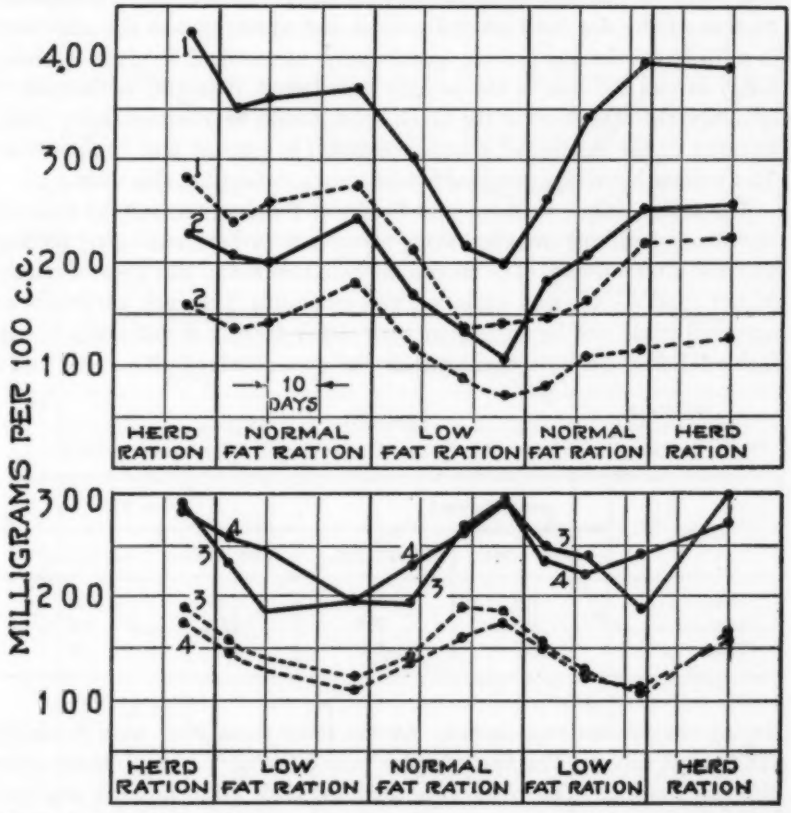

CHART 5. Fatty acids and cholesterol in blood plasma——— Fatty Acids– – – –Cholesterol.

throughout the period. The significance of these changes in blood lipids will be further discussed later.

In view of the fact that in the dog and other animals studied there is a marked rise in blood lipids following the ingestion of food containing fat, and the fact that constant values as well as a parallelism between fatty acids and cholesterol are found only in the post-absorptive state, the question naturally arises as to why the curves in Chart 5 are so uniform.

If the level of blood lipids in the cow is as greatly affected by food consumption as has been shown for the dog, then the results in Chart 5 would seem rather fortuitous and the significance that has been attached to them in the previous discussion would seem questionable. Since the normal ration of the cow contains only a small percentage of fat compared to that of the dog, and since digestion and absorption in the ruminant is a fairly continuous process, particularly where food is given 5 times a day as was the case in the present experiment, it seems, on theoretical grounds, that the level of the blood lipids would be comparatively little affected by the feeding of a ration uniform in amount and composition. The writers have experimental evidence to substantiate this view.

The senior writer, working with Professor Porcher, studied the lipids in the blood of milking cows by taking samples at various hours after feeding and also after milking. The maximum variation found was approximately 20 per cent, the average variation was much less, and such variations as occurred could not be correlated with either feeding or milking (14). In Table III there are presented some similar data obtained with cows 1 and 2

TABLE III

LIPIDS IN BLOOD PLASMA BEFORE AND AFTER FEEDING. MILLIGRAMS PER 100 CC.

Hour	Cow 1		Cow 2	
	Fatty Acids	Cholesterol	Fatty Acids	Cholesterol
5 A.M.	398	200	226	122
7 A.M.	380	206	223	124
11 A.M.	395	220	253	116

during the present experiment. At the time, these cows were receiving the normal ration. The first samples were taken at 5 A.M., 10 hours after the last feeding. The cows were then fed their grain mixture and two other blood samples were taken 2 and 6 hours later. The data show no rise in blood lipids following the intake of food and a maximum variation of around 15 per cent.

The results of the determinations of the iodine number of the milk fat are shown in Chart 6. The values represent determinations on the six-day composite samples of milk. The data show clearly that the iodine numbers were lower with the low-fat ration, indicating a distinct change in the character of the fat secreted. It is well understood from the work of several investigators reviewed by Terroine (15, p. 96) that the character

of the milk fat is influenced by the character of the food fat, that as the iodine number of the food fat increases, the iodine number of the secreted fat tends to rise. Engel (16) and Henriques and Hensen (17) have shown that inanition results in a rise in the iodine number of milk fat and Eckles and Palmer (18) have reported similar results for underfeeding.

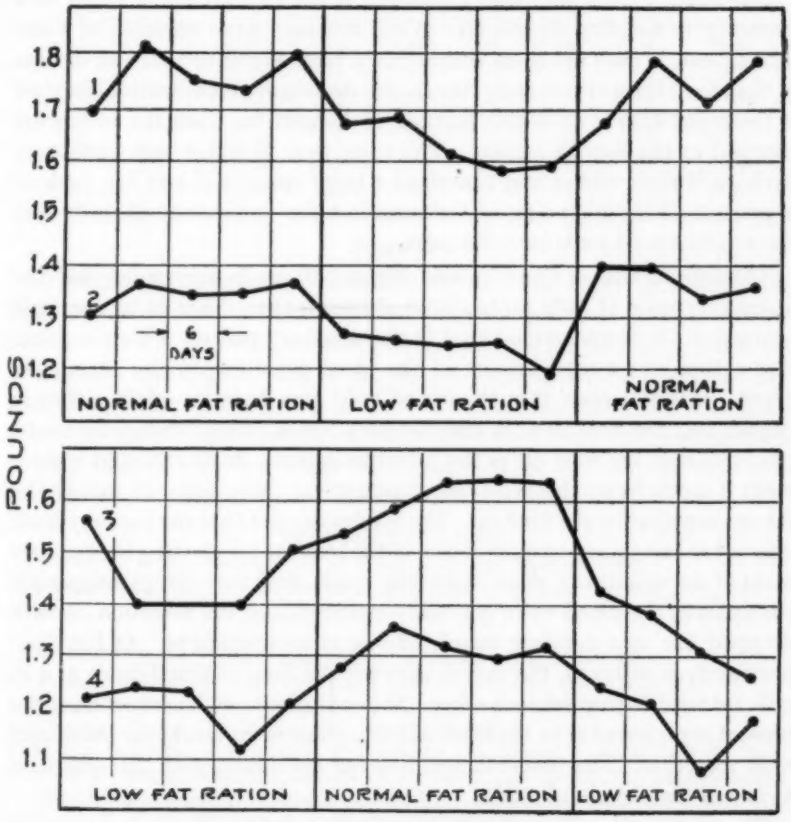

CHART 6. Iodine number of milk fat.

These findings coupled with the observation by Engel (16) and by Eckles and Palmer (18) that deposit fat is less saturated than milk fat indicate that the iodine number of milk fat rises in inanition because the deposit fat is being drawn upon for its manufacture. In the present study, however, the milk fat has a lower iodine number on the low-fat ration. It is understood from many experiments that when carbohydrate is substituted

for fat in a fattening ration, the iodine number of the deposit fat falls. It seems probable, therefore, that in the present experiment, where an adequate supply of energy was furnished, the animal when on the low-fat ration did not draw upon its reserves to any marked extent for a source of milk fat, but used instead the food carbohydrate for the purpose.

One might expect that with a lowering of the food fat there would be a tendency to mobilize deposit fat. While this may have occurred to a certain extent, it does not seem that it was a large factor or that the decline in the blood fat was primarily due to this decreasing mobilization, in view of the rapid drop in the iodine number of the milk fat when the cows were changed to the low-fat ration. The cows were in rather thin condition. Perhaps if their bodies had contained a large amount of soft fat, such as is present at the beginning of lactation in a cow previously liberally fed, the results would have been different.

The experiments of Foa (19) and Meigs (20) are in agreement that the blood precursor of milk fat is a lipid although they differ as to the lipid concerned. It is understood that in the secretory process a certain selective action and a modification of the blood fat occur in the mammary gland. It is also clear that this blood lipid may have its origin either in deposit fat or in food fat or in carbohydrate. On a ration rich in fat it seems probable that the food fat is the principal source. In the present experiment it seems likely that food carbohydrate was called upon to supply the fat not supplied by the food fat. The results suggest that the carbohydrate was not as suitable or useful a source as the food fat for meeting the requirement of the mammary gland, with the result that two things happened; the lipids in the blood were gradually reduced, and the secretion of milk dropped due to a deficient supply of one of its precursors. As has been previously mentioned, the curves showing the drop in blood lipids and in milk fat tend to parallel each other. No conclusions can be drawn from the present experiment as to the most suitable plane of fat intake for maximum milk secretion. The investigation is being continued with this question as one of the objects of study.

The same rations as used in the experiment with cows were fed to goats for periods of approximately 15 days and single determinations of the blood lipids and of the iodine number of the milk fat were made in the different periods. By themselves the data, shown in Table IV, are too meager to be of value, but it is significant that they show the same trend as the cow data and thus tend to confirm the conclusions drawn. They also indicate that goats may be suitable animals for a further study of this problem.

TABLE IV

LIPIDS IN BLOOD PLASMA AND IODINE NUMBER OF MILK FAT—GOATS

Goat	Ration	Mg. per 100 cc.		Iodine no. of milk fat
		Fatty acids in blood	Cholesterol in blood	
1	normal	169	93	26.0
	low fat	145	56	20.5
	normal	183	75	28.0
2	normal	193	97	30.0
	low fat	171	63	25.0
	normal	232	82	28.5

SUMMARY

Experiments to determine the influence of food lipids upon the milk secreted, have been carried out employing the cow and goat as experimental animals. A ration from which most of the fat had been extracted produced a marked lowering in the volume of milk secreted without any significant alteration in its fat content. The fat secreted in the milk upon a low-fat ration, had a lower iodine number then that produced upon a normal ration. The decreasing milk secretion on the low-fat diet was accompanied by a gradual decrease in the blood lipids.

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THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS
I. COMPOSITION AND HEAT VALUE

By

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THE nutritive value of cereal foods has been extensively studied in the past, but since the publication of Woods and Snyder's bulletin (1906) and Harcourt's paper (1907) there has been no general survey of the cereal breakfast foods in common use in comparison with one another and scarcely any study exists where the human subject has been used for comparison of these articles with other staple articles of diet. Meantime many new methods of study have been devised and new conceptions of food values have been acquired. The modern conception of the biological value of protein dates from 1909, and the entire, enormous literature on the vitamins has accumulated since 1912. Many conclusions regarding the vitamin values and protein values of cereal foods have been established by means of experiments on lower mammals; but there remains a considerable number of questions of nutritive value, both within and without these fields, which can be appropriately studied on the human subject. In the semi-scientific literature on foods and diets there are found not infrequently hints that the "super milling" and other processing, such as the sterilization with heat, steaming in preparation for rolling, crushing, toasting, crisping, and the like, to which our package breakfast foods are subjected, are really devitalizing in their effects and therefore destructive of important nutritive properties Is this a correct picture, or to what extent have the nutritive properties been affected by the modern milling and packing methods?

The opportunity to make a comprehensive study of this class of foods, which apparently has become a fixed element in the dietary of the American people, came in the form of a grant of money to this laboratory by one of the large producing companies a few years ago, the only stipulation on the part of the company being that their own product should be included in all phases of the study, and the stipulation on the part of the laboratory being that the results, insofar as they might be considered of general scientific interest, should be truthfully published. Aside from

this concern with the general question of the effects on the nutritive properties of this class of foods, the laboratory has had two other interests: (1) To determine what might be gained by newer methods of study, some of them originally devised for quite different purposes, and (2) to make a comparison of results obtained on the rat with results of the same kind of study on the human subject. The first of these interests related more particularly to the application of the Folin-Wu (1919) sodium tungstate method of precipitation of the proteins as an aid in the study of both protein and carbohydrate digestion in glassware, to the retention tube method of Rehfuss (1914), and Rehfuss, Hawk & Bergeim (1914) for studying gastric digestion, to the respiratory quotient as a measure of carbohydrate digestion and absorption, and to the dynamic action of a mixed carbohydrate and protein food in comparison with a nearly pure protein.

With reference to the second interest, a feeling has prevailed in certain quarters that nutrition studies have become rather too completely identified with the rat. There are still many respects in which human nutrition might be supposed to differ from that of the rodent. At all events, we need reassurance from time to time that what has been established for the rat is really true of man, so far, at least, as might be expected. Is the biological value of protein the same for the rat as for man, and is the rate of digestion and absorption comparable in the two organisms so widely separated in the zoological scale? We have not hoped to give a final answer to these questions but at least to make a start toward certainty as to the evaluation of nutritional studies on the lower mammals.

The entire program included studies of the following phases of nutrition. I. Digestion, both *in vitro* and *in vivo*. II. Absorption: (a) Of carbohydrate as indicated by the method of the R. Q. on the human subject and by the method of Rubner (1902) as adapted by Cori (1925) to the rat; (b) Absorption of protein as indicated by the method of nitrogen elimination; (c) Total utilization, meaning difference between intake and output by the stools. III. The biological value of proteins, as compared with the proteins of milk; (a) on the human subject; (b) on the rat. IV. The vitamins and proteins necessary for growth in the rat, as supplied by typical cereal breakfast foods. V. The dynamic action of cereal foods as compared with meat. The results obtained in these various phases of the study, so far as they have yet been carried, will be published in a series of papers of which this is the first.

The study presented herewith under the subtitle of "Composition and Heat Value" represents a part of the results of two summer's work by

several assistants. The first part of the title is not very completely treated, only a sufficient number of analyses having been carried out to reassure ourselves of the nature of the particular products being studied.

All of the cereals used in these studies on composition and digestibility were package goods. Comparatively little cereal breakfast food is now obtained in bulk by the American home. The advantage of protection against weevil by sterilization and hermetic sealing, now generally practised, not to speak of the appreciation of crispness and freshness in some products, insured by the moisture-proof package, is sufficient to offset the economic gain, except in institutional nutrition, of buying by the barrel. Analyses were not available for some of these package goods.

To avoid any appearance of discrimination as between the different cereal products included in the program, it has been considered best to use descriptive rather than trade names for the several cereals. What the science of nutrition seeks to make known are, first of all, the fundamental principles of nutrition and the education of the public must take care of particular choices, in accordance with these principles.

1. Composition

Six different, well-known breakfast foods have been used in this series of studies. They were chosen largely because of their popularity. Two are made from oats, one from corn, and three from the wheat grain. All of these were analyzed chemically and all but one were burned in a Kroecker bomb, the heat being measured with the Riche (1913) adiabatic bomb calorimeter. The first oat product is made from the whole grain, minus the hull, by precooking and crushing between rollers. The precooking is carried out to a different extent in different products sold under the same general trade name. The one used here is steamed for a few seconds only at 60 pounds pressure to sterilize and soften the grain preliminary to rolling and will be known simply as "Rolled Oats" in contradistinction from another product steamed longer, which will be called "Precooked Oats." The corn product is made from the decorticated and degerminated corn, is treated with a small amount of cane sugar and salt and cooked under steam pressure. It is then dried, rolled, and toasted on hot rollers. It will be called "Toasted Corn Endosperm." The first wheat product mentioned in the table likewise is made from the decorticated and degerminated grain but has nothing added to it and is only sterilized by heat, not cooked, before packing. We understand from the manufacturer that it represents that part of the wheat grain which would find its way into the so-called "patent white flour," merely diverted

from the process before it reaches the final rollers. It will be called "Wheat Endosperm." The second wheat product mentioned in the paper purports to be a whole grain product, but the analyses which we have obtained do not bear out this statement on the part of the manufacturer.[1] Because our analyses do not indicate that this product is truly the whole grain, we have decided to call it "Toasted Whole Wheat" using the expression in quotes. The third wheat product is labeled on the package "A Whole Wheat Cereal" but again our analyses indicate that the product does not contain the entire grain, because of the low fat content. This product however is not toasted, and therefore will be described simply as "Whole Wheat" using the expression again in quotes. From microscopic examination it is evident that both of these last named wheat products do contain a liberal amount of bran and the second contains a liberal amount of the wheat germ also.[2]

From this statement of the mode of manufacture of each of the products under examination, we are prepared for the differences in composition presented by Table I. The two oats preparations contain the highest percentage of protein and of fat, and correspondingly less carbohydrate (starch), because they consist of the whole grain. The Toasted Corn Endosperm, because it is dried by toasting and immediately packed in wax paper, contains, as one would expect, a very small amount of moisture as it reaches the consumer. It is correspondingly high in total solids, but because the corn is degerminated, and also because sugar and salt are added, it contains very low percentages of protein and fat. The Wheat Endosperm may be described as the whole grain less the germ and bran. Nothing having been added, the percentage composition is what nature intended for the endosperm. The "Toasted Whole Wheat" has a considerably lower moisture content than the non-toasted product just mentioned, as would be expected because of the effect of heat. Curiously enough, however, it has a lower percentage of protein and of fat

[1] The statement of the manufacturer is as follows: "We retain as nearly as possible, with careful chemical analyses, the exact proportions of the various parts of the wheat which constitute the wheat mix on the mill at the time. In other words, we retain the endosperm, the aleurone layer, the episperm, the testa, the endocarp, the epicarp, the epidermis and the germ, or heart. The chemical analysis of the ground whole wheat product which we use is somewhat changed in our process, which is one of toasting with nothing added and nothing taken away."

[2] It is a curious custom amongst millers as well as amongst consumers that has sanctioned the term "whole wheat" to indicate something considerably less than the whole wheat. To be sure of getting truly whole wheat the consumer must ask for "Graham" flour. The Food Standards Committee, appointed by the U. S. Dept. of Agriculture, has proposed recently some clarifying definitions of whole wheat flour, entire wheat flour and bolted Graham flour. Similar clarifications should be applied to the cereal breakfast foods.

than the wheat endosperm alone. The "Whole Wheat" product, because of careful packing, has a low moisture content and because it contains a considerable amount of germ and bran has a high protein content, but the low fat percentage indicates that some of the germ has been removed, for again the percentage is lower than that of the Wheat Endosperm. Some allowance must be made here for different varieties of wheat and wheat grown under different conditions. The recent paper by Greaves and Hirst (1929) has sharply called attention to the wide variations in mineral content of oats, barley, corn and wheat grain grown on different soils and with and without irrigation, in the State of Utah. Similar studies are very much needed with reference to the effect of soil and particularly of fertilizers on the other nutritive properties of the cereal grains.

TABLE I
COMPOSITION (Average Percentages)

	Moisture	Total Solids	On Dry Basis Ash	Protein N x 6.25	Fat (ether extr.)	Carboh. (by diff.)
Rolled Oats	11.02	88.98	2.06	17.63	8.13	72.18
Precooked Oats	7.36	92.64	1.84	18.43	6.14	73.59
Toasted Corn Endosperm	7.49	92.51	3.07*	7.98	1.82	87.13**
Wheat Endosperm	12.68	87.32	0.74	13.52	2.75	82.99
"Toasted Whole Wheat"	7.06	92.94	1.35	12.99	1.89	83.77
"Whole Wheat"	8.08	91.92	1.45	16.49	1.47	80.59

* Some NaCl added.
** Some Sugar added.

The table presents the average percentage composition of the six cereal breakfast foods used in the following studies on the dry basis so as to make them comparable in spite of their varying moisture contents found in the package.

2. HEAT VALUE

How a food ranks as a source of energy can be determined approximately by burning it in pure oxygen and measuring the heat given off. With all protein foods an allowance must be made for that part of the protein molecule which is split off as ammonia and becomes converted to urea before its elimination from the body; in other words, for the fraction not oxidized. This correction alone, however, does not give the true physiological heat value, for a considerable fraction of the organic constituents may not be absorbed. This is true of the protein of cereals,

particularly if they are consumed as whole cereal. The only practicable way to obtain the heat value to the body of any article of food is to determine the percentage utilization (intake minus excretion by the feces) for each of the organic foodstuffs and then to calculate its utilization or metabolizable energy from the percentage composition, using the established heat values for the kind of protein, fat and carbohydrate ingested in that article. Atwater and his associates (1901, 1903) elaborated a method for this calculation, which is not sufficiently known, and may with propriety therefore be described here very briefly.[3]

The percentage utilization is obtained from analysis of the feces for protein, fat and carbohydrate, the difference between these figures and those representing the composition of the food itself (both on dry basis) being expressed as percentages of the original components. Knowing these utilization coefficients, so-called, a utilization or metabolizable heat value per gram of protein, fat and carbohydrate is obtained by multiplying the average calorimetric heat value of the kind of protein, fat and carbohydrate ingested by the utilization cœfficients, and in the case of protein making a further correction for the heat value of a gram of nitrogen in the urine.[4] For example, the average bomb value of one gram of vegetable protein as found by the Atwater school is 5.8 Cal., for vegetable fat 9.4, and for carbohydrate 4.2 Cal. In the case of Rolled Oats, as determined in the present studies (see paper IV of this series) the utilization coefficient is 84 per cent (Table II) for protein, 90 per cent[5] for fat, and 96.6 per cent for carbohydrate. The utilization heat values therefore are 3.82 Cal. for the protein, 8.46 Cal. for the fat and 4.06 Cal. for the carbohydrate in Rolled Oats (Table II). The amount of heat obtained by the body from one gram of Rolled Oats (dry) is then obtained by multiplying these heat values by the amount of protein, fat and carbohydrate in a gram of the cereal (See Table I) and adding them together.

Comparison of the physiological heat values for the different cereal products with their calorimetric or combustion heat values is instructive. The latter were obtained by burning the cereals, after drying to constant weight or as obtained from the package. The directly determined values are given in the table in italics; the calculated values on dry or moist basis, from knowledge of the moisture content, are given in ordinary

[3] For a fuller statement the reader is referred to the senior author's review of the subject in Endocrinology and Metabolism, New York, 1922, Vol. III, p. 551.

[4] The factor for the heat value of a gram of nitrogen in the urine is 7.9 Cal. or for a gram of protein represented by this nitrogen (7.9÷6.25) is 1.25 Cal.

[5] Not determined in these studies but adopted from Atwater (*loc. cit.*) for cereal foods generally.

TABLE II

HEAT VALUE OF CEREAL FOODS BY BOMB CALORIMETER COMPARED WITH PHYSIOLOGICAL HEAT VALUE

Cereal Food	Heat of Combustion		% Moisture	Physiological Heat Value Based on Percentage Utilization* & Composition (dry basis)**			
	Dry Basis Cal. per gm.	Moist Basis Cal. per gm.		Coefficient of Utilization	Calories per Gram		Loss
					Component	Cereal Food	Cal. per gm.	Per cent
Rolled Oats	*4.761*	4.320	9.27	P. 84.	3.82	0.673		
	4.807	4.361	9.27	F. 90	8.46	0.688		
	4.794	*4.371*	9.68	C.H. 96.6	4.06	2.928		
	Av. 4.787	4.351			Total	4.289	0.498	10.4
Toasted Corn Endosperm	*4.155*	3.857	7.16	P. 84	3.82	0.305		
	4.159	3.861	7.16	F. 90	8.46	0.154		
	4.218	*3.894*	8.34	C.H. 97.1	4.08	3.555		
	4.186	*3.864*	8.34					
	Av. 4.179	3.869			Total	4.014	0.165	4.1†
Wheat Endosperm	*4.432*	3.966	10.50	P. 94	4.28	0.578		
	4.394	3.833	10.50	F. 90	8.46	0.233		
	4.430	*3.970*	11.63	C.H. 96.3	4.04	3.353		
	Av. 4.414	3.923			Total	4.164	0.250	5.6
"Whole Wheat"				P. 87.2	3.97	0.655		
	4.440	4.084	8.08	F. 90	8.46	0.124		
	4.423	4.065	8.08	C.H. 96.	4.02	3.240		
	Av. 4.431	4.074			Total	4.019	0.412	9.3

* As determined in these studies (see paper IV). ** See Table I.

† This low value is due in part to the added sugar.

type. Since the physiological value was calculated on the dry basis the comparison with crude or bomb heat value can be made only on this basis.

For the Rolled Oats the loss in heat value from non absorption and non combustion (of protein) is 0.498 Cal. per gram or 10.4 per cent. For the Toasted Corn Endosperm the loss is 0.165 Cal. per gram or 4.1 per cent; for Wheat Endosperm, 0.250 Cal. per gram or 5.6 per cent; and for "Whole Wheat", 0.412 Cal. per gram or 9.3 per cent. The whole grains, or nearly whole grains, obviously lose more than the endosperm products on account of the low digestibility of the bran.

The calorimetric heat value of the Rolled Oats is highest because of its higher fat content, the "Whole Wheat" is next in order, but because its fat content is lower than that of the Wheat Endosperm, it ranks only a little higher than the latter. The Corn Endosperm is lowest of the four cereal products compared. Only one of the oats preparations was burned in the bomb, because it was assumed that the Precooked Oats would have the same heat value as the regular Rolled Oats. The chemical analyses given in Table I do not quite justify this assumption, although the difference is not great. The "Toasted Whole Wheat" was burned and was found to have almost identically the same heat value as the "Whole Wheat" not toasted. It is not included in Table II, because the data for calculation of the physiological heat values were not available.

For actual value to the body on the basis of these studies the Rolled Oats ranks first, the "Wheat Endosperm" next, and the Toasted Corn Endosperm and "Whole Wheat" tie for third place.

The nutritive value of the different preparations, however, may not follow exactly this order because of differences in *rate of digestibility*. The following paper deals with this factor.

Summary

Six different cereal breakfast foods, three from wheat, two from oats and one from corn, all widely used in the United States, have been analyzed, all but one burned in the bomb calorimeter, and their physiological heat values compared with the combustion heat values in four, as a preliminary to a more detailed comparative study of nutritive values.

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THE NUTRITIVE VALUE OF CEREAL BREAKFAST FOODS
II. DIGESTIBILITY IN VITRO, WITH A STUDY OF METHODS

By

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THREE different studies were made of the digestibility in glassware of the cereal breakfast foods whose analyses and heat values have been described in the preceding paper. In the first only three of the foods were used, namely, the Rolled Oats, the Toasted Corn Endosperm and the Wheat Endosperm.

FIRST STUDY

The method adopted in this study has, so far as we are aware, not been employed hitherto. It consisted essentially in carrying the same materials through a digestion by salivary amylase, pepsin-HCl, trypsin and diastase, successively.

Method 1. Mouth Insalivation and Successive Digestions on the Same Sample

Fifty-gram samples of each cereal were weighed into beakers. The wheat and oats products were boiled in 400 and 300 cc. of water, respectively, containing a little salt. The corn product, being already dextrinized by toasting, was not cooked. The cooked cereals were then transferred to weighed beakers and the cooking vessel washed out, the washings being added. Aliquot duplicate samples were next taken of all three cereals and set aside as controls. The rest of the cereal was taken into the mouth and chewed until ready for swallowing and then was expectorated into weighed beakers. The entire quantity thus prepared for salivary digestion was then divided by weighing by difference into 14 nearly equal portions, placed in as many smaller beakers, the volume of each being made up to 78–80 cc. All were incubated together for two hours at body temperature, after which two were heated to stop the action of ptyalin. To all the others HCl and pepsin were now added. Two of these were immediately heated to stop the action of pepsin and the balance incubated for two hours at body temperature, and so on.

Table I exhibits the entire scheme of treatment. In the case of each enzyme there were two control vessels in which digestion was not allowed to proceed and two in which digestion continued for two hours with stirring at half-hour intervals. The amount of digestion of starch by ptyalin, for example, then was determined by the difference in the amount of sugar formed in the filtrates of the digested as compared with the control lots. Sugar was determined by Benedict's (1911) quantitative method and calculated as glucose. As a further check on this method the residual starch left on the filter paper after successive washings to remove sugar was digested in 2.5 per cent HCl under reflux for two and a half hours and the sugar from this digestion determined in the same manner. The amount of sugar found in the filtrate after hydrolysis, expressed as starch, should agree with the difference between the original starch and that found (as sugar) after digestion.

TABLE I

PARTITION OF EACH CEREAL FOR CONTROLS AND FOR DIGESTION SUCCESSIVELY BY DIFFERENT ENZYMES

Samples.

1 2 Set aside before chewing as controls

Remainder of each cereal chewed thoroughly, expectorated into weighed beakers, divided into 14 nearly equal portions (by weighing by difference) as follows:

3 4 5 6 7 8 9 10 11 12 13 14 15 16 All digested in incubator 2 hrs. at 38–39°C. Shaken every half hour.

1 2 3 4 Boiled 3 and 4 several min. to stop ptyalin. Washed all 3 times by decantation, filtered, sugar determined in filtrate; residue digested with 2.5% HCl and sugar from residual starch determined.

5 6 7 8 9 10 11 12 13 14 15 16 Added 10 cc N · HCl + 5 cc pepsin solution.

5 6 Boiled at once to stop pepsin

7 8 9 10 11 12 13 14 15 16 Incubated 2 hrs. at 38–40°. Shaken every half hour.

7 8 Boiled after 2 hrs. incub.

9 10 11 12 13 14 15 16 Added 4 cc 2.5 N · NaOH to neutralize acid then 10 cc trypsin-diastase solution in 0.1 N · NaOH

5 6 7 8 Precip. with 2.5% trichloracetic, stood 2 min., heated to boiling, filtered hot, washing residue hot water, N. detn. on filt.

9 10 11 12 Heated at once to stop trypsin & diastase.

13 14 15 16 Incub. 2 hrs. 38–40° then heated.

9 10 Controls for trypsin

11 12 Controls for diastase

13 14 Precip. with trichloracetic

15 16 Washed by decantation.

9 10 11 12 Same method for tryptic digestion as for peptic.

13 14 15 16 Same method for diastase as for salivary amylase.

In following the proteolytic digestion by pepsin and trypsin, as will be noted in Table I, comparison was made between the samples heated at once after the addition of the enzymes and similar samples heated after digestion for two hours. The undigested protein was then precipitated by means of trichloracetic acid, the mixture being heated to boiling after standing for two minutes and filtration immediately following while the mixture was still hot. The residues on the filter paper were washed with hot water several times, the filtrate cooled and made up to volume after which nitrogen was determined on an aliquot sample.

This method, while especially satisfactory from the standpoint of economy of time and as a means of imitating the proper order of digestion in the alimentary tract, did not always give satisfactory comparisons. The reasons probably are amongst the following. 1. It is difficult to secure exactly equal salivation in cooked and uncooked cereals and of two different

cooked cereals of wholly different physical characteristics. 2. The division of the cooked and chewed cereal into equal portions by difference by weight is not very exact because of the difficulty of maintaining homogeneity in the suspension during weighing operations. 3. Determination of starch digestion for assay of sugar in the filtrates containing a considerable amount of protein was not wholly satisfactory. For these reasons the results of this first study will not be given in detail. We believe that with suitable precautions, and some slight modifications, the method can be made very serviceable.

The results indicated that the Toasted Corn Endosperm gave the largest percentage of digestion of starch in two hours, the Rolled Oats next, and the Wheat Endosperm third. Peptic digestion, however, was best in the wheat product, second in the oats, and third in the corn product. The already soluble carbohydrate in the toasted corn product was, of course, properly controlled and the same is true of the soluble proteins present in considerable amount in both the corn and the oats products (Harcourt 1907).

Second Study

In the second study the difficulties encountered in the first were overcome to a large extent by weighing the individual samples separately and cooking them separately in individual double glass boilers, consisting of two beakers of the same shape. Saliva was collected from several different individuals, mixed, filtered, and added in equal amounts to the several samples. It was desired in this study also to observe the difference due to different lengths of digestion and also the effects of cooking different lengths of time. Consequently the entire number of samples of a single cereal started simultaneously was increassed to twenty-four. The order of digestion and the general treatment of the product in each case was similar to the outline already exhibited in Table I, that is to say, peptic digestion followed salivary digestion, tryptic digestion followed peptic digestion, and digestion by pancreatic diastase occurred simultaneously with the tryptic digestion.

Method 2

Samples 1 to 6 were distributed as follows; 1 and 2 for controls, 3 and 4 for salivary digestion one hour, 5 and 6 for salivary digestion two hours. Samples 7 to 12 likewise were distributed as follows: 7 and 8 served as controls, 9 and 10 peptic digestion for one hour, 11 and 12 peptic digestion for two hours. Samples 13 to 18 inclusive were distributed in similar manner for tryptic digestion. Samples 19 to 24 inclusive similarly for pancreatic diastase.

Under favorable conditions, and with plenty of assistance, it is possible to put one cereal of a single cooking through the entire program of digestion in a single day, but as a rule it was

necessary to stop digestion by heating at some intermediate point as, for example, just before pancreatic digestion, and to leave it in this condition over night.

It was found that the tendency of the cereals to form a crust after cooking was a serious obstacle to uniform admixture with the saliva unless the cereals were stirred thoroughly during the process of cooking. As in the first, so in this study, no attempt was made to stop the action of ptyalin by heat in the samples which were to be continued under peptic digestion, but to depend upon the addition of hydrochloric acid for this purpose.

For peptic digestion Merck's powdered pepsin was used in 2 per cent solution. This was added to each of the beakers No. 7 to 24 in sufficient amount to make 0.17 grams of pepsin in the digestive mixture, following the addition of HCl to a concentration of one-tenth normal. Beakers 7 and 8 received the enzyme and then were immediately heated to stop its action. Beakers 9 and 10 were removed from the incubator at the end of one hour and were heated. Beakers 11 and 12 were removed at the end of two hours and heated. Beakers 13 to 24 at the end of two hours received 10 per cent sodium hydroxide in sufficient amount to neutralize hydrochloric acid.

Before addition of pancreatic enzymes, the samples were allowed to stand following neutralization for at least one-half hour to allow for change of reaction due to amphoteric properties of the cereal protein. The reaction was corrected, if necessary, to the neutral point after which sodium carbonate solution was added in sufficient amount to make the mixture, increased in volume, contain 0.3 per cent of the alkali. Fairchild Bros. and Foster's *Extractum pancreatis* was used in 4 per cent solution and a sufficient amount added to each of the beakers 13 to 24 to make 0.4 per cent of the dried extract. Beakers 13 and 14 were immediately heated to serve as controls for tryptic digestion. Beakers 19 and 20 served as controls for diastase digestion. The entire twelve remaining beakers were then placed once more in the incubator and after one hour beakers 15 and 16, 21 and 22, were removed and heated to above 80°. At the end of two hours the remaining four beakers were heated likewise to above 80°.

Separation of the soluble portions from the undigested residue after starch digestion was accomplished by centrifugation. The supernatant fluid was perfectly clear for the wheat preparations and only slightly turbid for the oats preparations. In the pancreatic samples (19 to 24) on the other hand, there was a thin milky suspension due to the small amount of fat set free by proteolytic digestion and not split by lipolytic digestion. This, however, did not interfere with the determinations either of sugar or nitrogen.

Sugar was determined in the supernatant fluids by a modification of Bertrand's (1906) method. The modification consisted of separating the copper oxide by centrifugation rather than by filtration through a Gooch crucible, washing the copper oxide once with distilled water and centrifugation again, after which the oxide was dissolved in the ferric sulfate-sulfuric acid solution. This solution was titrated in the usual manner with permanganate.

In this second study two wheat products and two oats products were used. The first wheat product was the one described in the first study under the name of Wheat Endosperm. The second was the "Whole Wheat" ground to about the same degree of fineness as the Wheat Endosperm preparation.

The second oats preparation was the same commercial brand as the one described in the first study under the name Rolled Oats but was precooked. For this reason it will be described simply as Precooked Oats.

The results of this method of study were again not wholly satisfactory, probably because salivary amylase is not wholly inactivated by heating

to boiling temperature (Gramenitski, 1910; Biedermann, 1914–1916). Boiling had been used to arrest the action of ptyalin immediately after addition of saliva also at the end of one hour and of two hours. We were not aware of this surprising stability of ptyalin at the time this part of the work was done. The second cause of discrepancy probably consisted in the unequal separation of partially digested (that is, soluble) starch from residue by centrifugation. That a considerable amount of soluble starch was present in the supernatant fluid was demonstrated by hydrolysis with hydrochloric acid. The two fractions, reducing sugar before hydrolysis and reducing sugar after hydrolysis, were determined separately.

The determination of sugar by the Bertrand method of supernatant fluids was satisfactory in spite of the small amount of soluble protein present. This was confirmed by a precipitation of the proteins, where they occurred in sufficient quantity, by means of 10 per cent sodium tungstate. Precipitation of proteins for determination of the proteolytic digestions was accomplished, as before, by precipitation with trichloracetic acid. The washing of the precipitate as in the first study did not always yield good duplicates.

For the several reasons enumerated above, the results of this study will not be reported in detail. They indicated that Wheat Endosperm showed practically the same digestion of starch under salivary amylase at the end of one hour and of two hours as did the Precooked Oa s. The "Whole Wheat" preparation gave similar percentages at both intervals and the Rolled Oats preparation gave the least percentage of digested starch at both intervals. These results were reached by both methods mentioned under the first study; namely, first, direct determination of the total sugar in the supernatant fluid, after hydrolysis and centrifugation, calculated as starch, and second, hydrolysis of the residual starch and subtraction of the resulting sugar, calculated as starch, from the original starch content. Not quite the same order of results was obtained after cooking for one hour as for cooking for fifteen minutes. The results of the proteolytic digestions were a little irregular in that the amount of digestion obtained after one hour of cooking was less in several instances than after fifteen minutes of cooking. The results indicated, however, that the two wheat preparations were about on a par, one with the other, both under peptic and tryptic digestion. The two oats preparations also did not differ widely under peptic digestion but showed a surprisingly larger percentage of digestion under trypsin after cooking for one hour in the Rolled, as compared with the Precooked Oats.

Third Study

It was thought best to separate the digestion by different enzymes rather than to use the enzymes in succession on the same samples as in the two previous studies. Cumulative errors in the different stages of digestion would thereby be avoided. In this study also we were interested again in the effect of cooking time upon digestibility as well as in the progress of digestion at different intervals of time. Consequently to follow the effect of different enzymes in succession on the same sample would require too many samples at the start to be readily manageable. As will be seen in the tables which follow, the partial digestion of starch all the way to reducing sugar, as compared with total starch made soluble, was studied. With the proteolytic enzymes the criterion of digestion was the amount of protein made filterable through a given filter paper without reference to whether this protein in solution was in the form of proteoses or final split products.

The third wheat product, "Toasted Whole Wheat" was introduced in the third study. This, like the other cereals is a package product differing from the "Whole Wheat" apparently only in the fact that it was toasted before packing. The chemical analyses, however, show that it contains less protein and slightly more fat. These differences may well be due to the kind of wheat selected.

A. Salivary Digestion

The procedure for study of salivary digestion was as follows: The cereals were cooked in a single boiler for different lengths of time, cooled to body temperature, mixed with saliva, and then subjected to incubation up to a maximum of two hours. In the beginning, only 3 grams of each cereal were used, and at first 10 cc. of saliva. In all cases saliva was collected from only two individuals and these two salivas were mixed in equal proportion. After the preliminary experiments, a large quantity of saliva was collected over a period of several days from the same two subjects, mixed in equal proportion, preserved with chloroform, and placed in the ice box. Thereafter, until the very last experiments of the series, saliva was used from this stock supply, and was therefore uniform in diastatic enzyme and in other qualities.

In the first two series of experiments in the third study the small beakers, which served as single boilers, after cooling to body temperature, were placed in a large incubator room for periods of 1 hour and 2 hours, after which the beakers were heated to boiling temperature to arrest the

ptyalin. The contents of the beakers were then washed out, diluted and filtered or centrifuged, and sugar determined on the filtrate or supernatant fluid.

TABLE II

AVERAGE PER CENT STARCH DIGESTED COMPLETELY TO SUGAR BY

Digestion for — — —	5 min.		1 hr.		2 hrs.	
After cooking — —	5 min.	15 min.	5 min.	15 min.	5 min.	15 min.
Wheat Endosperm	19.4	22.7	33.6	32.0	35.0	34.4
Precooked Oats	38.7	34.9	47.6	47.1	52.4	50.3
"Whole Wheat"	23.2	26.8	37.4	40.9	41.0	42.9
"Toasted Wh. Wheat"	30.1	31.1	49.1	52.4	51.9	55.5

Tables II and III illustrate these experiments. The results are averages of from two to four experiments with duplicate determinations for each. The sugar actually formed by action of the ptyalin of saliva is, of course, maltose and not dextrose, for, according to most authorities (Punnett, 1915; Blake, 1916), a very little dextrose appears in the digestion even of soluble starch by animal or vegetable amylases. The advantage of expressing the results in terms of dextrose, however, is that it permits a direct comparison of the starch immediately transformed into sugar with that only partially transformed or changed far enough to pass through filter paper or escape precipitation by tungstate.

TABLE III

AVERAGE PER CENT STARCH DIGESTED FAR ENOUGH TO PASS THROUGH FILTER PAPER BY

Digestion for — — —	5 min.		1 hr.		2 hrs.	
After cooking — —	5 min.	15 min.	5 min.	15 min.	5 min.	15 min.
Wheat Endosperm	59.8	70.7	84.1	82.6	85.2	87.6
Precooked Oats	76.8	77.2	82.1	86.0	83.9	88.0
"Whole Wheat"	69.6	67.6	87.5	73.8	90.6	85.3
"Toasted Wh. Wheat"	62.5	66.8	88.4	89.8	90.2	89.7

Later it was learned that a smaller amount of saliva sufficed to give a sufficient amount of digestion in a short space of time for accurate measurement, and more accurate temperature control was secured by use of a small incubator.

Referring now to the results of the comparison of different cereals in their behavior to different lengths of time of boiling, we see from the average figures in Table II that when the cereals were cooked 5 minutes

and 15 minutes, so far as the immediate formation of sugar is concerned, the Precooked Oats heads the list followed by the "Toasted Whole Wheat" and "Whole Wheat" and Wheat Endosperm in that order. When, however, the filtrate was hydrolyzed with hydrochloric acid, converting not only maltose but also soluble dextrins to dextrose and then analyzed, it was found (Table III) that all four cereals stand on practically the same footing. The surprising thing in this study is that from one half to nearly four-fifths of the digestion accomplished in 2 hours takes place in the first five minutes. We shall have more to say about the importance of this observation later.

The figures for percentage of starch completely changed into sugar in 5 minutes of digestion, after 15 minutes cooking, as compared with 5 minutes cooking, are raised a little for the Wheat Endosperm, the "Whole Wheat" and the "Toasted Whole Wheat," but not for the Precooked Oats. When digestion goes on for as long as 1 hour or 2 hours there is no advantage in cooking 15 minutes over cooking 5 minutes for the Wheat Endosperm or the Precooked Oats, but there is some advantage for both "Whole Wheat" preparations.

Method 3

In the course of the second study trial was made of sodium tungstate as a means of precipitation of the soluble proteins in the supernatant fluids. In the third study it occurred to us to use sodium tungstate as a means of precipitation of both protein and non-digested starch. We were encouraged in the belief that this reagent would prove useful as a means of securing protein-free filtrates by the experience of Rumsey (1922) in his study of wheat flours. In the preliminary trials with this method we were led to suspect the stability of saliva to heat and on search of the literature discovered Biedermann's paper, referred to above, confirming this observation. The well-known rapidity of the action of ptyalin also led us to make a further modification providing for the dumping of equal quantities of mixed saliva into the several beakers inside the incubator, simultaneously, so that digestion might be started at the same instant in the several preparations. The same mechanical device providing for the tilting of test tubes containing the saliva, served also for dumping equal quantities of the sodium tungstate into the several beakers simultaneously to stop salivary digestion. This simultaneous starting and stopping of digestion in one operation proved to be in importance second only to the use of sodium tungstate as a precipitant and both improvements together enabled us to secure perfectly satisfactory duplicate determinations in all of the cereals and under all conditions employed with the different enzymes.

The use of sodium tungstate had to be adapted to the reaction and different physical conditions of the cereal breakfast foods and therefore suffered considerable modification from the conditions found most satisfactory by Rumsey. We found 15 per cent sodium tungstate appropriate but at times it was necessary to use alcohol in conjunction with the tungstate to secure a perfectly clear filtrate upon which satisfactory sugar or nitrogen determinations could be made. Also, it was found necessary to change the reactions considerably from that found most favorable by Rumsey. Indeed, each cereal required a different reaction. The use of this method, however, made it possible to employ Benedict's quantitative method for sugar, which is considerably more rapid than the method of Bertrand and just as accurate in water-clear filtrates with no interfering substances present.

Tables IV and V give the results of simultaneous experiments after cooking 30 minutes and 1 hour, respectively, where the four cereals were used in parallel lots. The digestion for 15 minutes and 1 hour, however, were run on separate days and really constitute separate and independent experiments.

TABLE IV

AVERAGE PER CENT STARCH TRANSFORMED TO SUGAR BY

Digestion for — — —	15 min.		1 hr.	
After cooking — — —	30 min.	1 hr.	30 min.	1 hr.
Wheat Endosperm	17.4	25.9	22.4	29.8
Precooked Oats	38.6	38.5	44.0	45.8
"Whole Wheat"	24.7	38.7	31.6	42.0
"Toasted Whole Wheat"	44.1	43.6	47.5	51.1

By cooking 30 minutes, as may be seen in Table IV the digestibility of the starch in Wheat Endosperm is 17.4 per cent, in Precooked Oats it is 38.6 per cent, in "Whole Wheat" 24.7 per cent, and in "Toasted Whole Wheat" 44.1 per cent. All of these figures are for digestion only 15 minutes.

In the same summary table are presented, the results after cooking one hour. Digestibility in 15 minutes' exposure to saliva is raised for Wheat Endosperm from 17.4 per cent after cooking 30 minutes to 25.9 per cent after cooking one hour, a relative increase of 50 per cent. The digestibility of the oats product is scarcely improved. The same is true of the "Toasted Whole Wheat" but with the "Whole Wheat" digestibility is raised from 24.7 per cent after cooking 30 minutes to 38.7 per cent after cooking 1 hour, or again, a relative increase of 50 per cent.

In Table IV are presented also the data for sugar formation after 1 hour of digestion. It may be noticed that carrying digestion only 15 minutes yields fully four-fifths of the quantity of sugar produced in one hour, whether cooking is for 30 minutes or one hour. In other words, the amount of sugar produced by ptyalin is not at all proportional to the time of exposure. Biedermann (1914–1916) has called attention to the almost "explosive" character of the action of saliva on starch paste. The action on the starch of cooked cereals is not exactly *explosive*, but may be described as *very* rapid.

Much less benefit of cooking 1 hour as compared with 30 minutes is seen when we lay side by side the results of digestion for one hour. The Wheat Endosperm gains 7.4 per cent, the Precooked Oats 1.8 per cent,

the "Whole Wheat" 10.4 per cent, and the "Toasted Whole Wheat" 3.6 per cent. The two precooked foods gain less than the raw foods.

TABLE V
AVERAGE PER CENT STARCH TRANSFORMED FAR ENOUGH TO ESCAPE PRECIPITATION WITH TUNGSTATE

Digestion for — — —		1 hr.
After cooking — — —	30 min.	1 hr.
Wheat Endosperm	77.2	80.9
Precooked Oats	76.1	80.5
"Whole Wheat"	77.7	84.3
"Toasted Whole Wheat"	85.4	90.7

On the other hand, the percentage of total starch transformed far enough to escape precipitation with sodium tungstate and existing as water-clear filtrate is raised to about the same extent by cooking one hour, as compared with cooking 30 minutes, for all four cereals (Table V). Precooking, therefore, affects only the digestion to sugar.

We may summarize all the results on salivary digestion briefly in the following statements: After cooking 5 minutes the starch and dextrins of all of the cereal breakfast foods studied are transformed to maltose to the extent of 20 to 40 per cent after exposure to saliva at body temperature for only 5 minutes. They are digested far enough to be in perfect solution to the extent of 60 to 75 per cent by exposure for only 5 minutes. After cooking for only 5 minutes the starches and dextrins of all of the cereal breakfast foods studied are transformed to maltose by exposure to saliva for 1 hour to the extent of 35 to 50 per cent. They are digested far enough to pass through filter paper and form a perfect solution after the same length of digestion to the extent of 82 to 88 per cent. These figures are but little changed by exposure to saliva for 2 hours. In other words, the digestion of starches and dextrins in the cereal breakfast foods would be nearly complete at the end of 1 hour's exposure to saliva.

Using 5 to 10 cc. of saliva to 3 grams of cereal represents very thorough insalivation, such as would take place in prolonged chewing of the cereal and digestion in the mouth. Saliva acts so rapidly that thorough mastication is of more importance than longer cooking (cf. Day, 1908). Boiling 30 minutes instead of 15 minutes improved digestibility only 3 or 4 per cent in absolute terms. Relatively, however, this amounts to from 10 to 30 per cent. One reason for this is the tendency of the cereals to clump or gelatinize and stick together while actively boiling, as will be emphasized

more especially under the head of tryptic digestion. Boiling for one hour as compared with boiling for 30 minutes, improves complete digestibility for 15 minutes' exposure more than 50 per cent, in relative terms, for Wheat Endosperm and "Whole Wheat," the two cereals which are not precooked, but not at all for the Precooked Oats and the "Toasted Whole Wheat" the two which are precooked. It improves partial digestibility; that is, the conversion of starches and dextrins to the point where they escape precipitation with tungstate to the extent of only 5 to 10 per cent relative, for any cereal.

In general it is clear from this study on salivary digestion that cooking any of the cereals for 5 minutes gives a very good start to the utilization of the energy contained in cereal foods *in the form of starch.* Previous dextrinization facilitates this earlier transformation enormously.

B. Peptic Digestion

The general procedure for the study of the action of pepsin on the cereal breakfast foods was as follows: 5 grams of each cereal were weighed out, placed in a small beaker with 80 cubic centimeters of water and boiled over a hot plate for the requisite length of time, after which the cereal was cooled to body temperature, 20 cc. of N/10 HCl added, and the volume made up to 100 cc. The beakers were then set in the incubator in such a position that eight cubic centimeters of pepsin solution could be dumped simultaneously into all. Incubation then proceeded for the proper length of time at the expiration of which digestion was stopped in all the beakers by dumping 25 cc. of 15 per cent sodium tungstate solution simultaneously from test tubes into all the beakers. After filtration, nitrogen determinations were made on the filtrates.

A considerable amount of soluble nitrogen already exists in cereals, particularly cereals that have been cooked. Hence it was necessary to determine how much nitrogen could be obtained in filtrates from sodium tungstate precipitation of the cereals after cooking but without digestion. After deducting the soluble nitrogen present in the pepsin solution itself there remained, after boiling 15 minutes, 14.1 per cent soluble nitrogen in Wheat Endosperm, 12.3 per cent in Precooked Oats, 14.3 per cent in the "Whole Wheat" and 18.1 per cent in the "Toasted Whole Wheat." The results were the same after boiling 30 minutes and 1 hour except in the case of the Wheat Endosperm which showed an increase of soluble nitrogen after cooking for 1 hour. Deduction of these amounts expressed as percentages of the total nitrogen present in the cereals has been made in the following tables.

A preliminary study of peptic digestion showed that very little additional soluble nitrogen could be obtained after digestion for only 5 minutes, hence the smallest time used for digestion in this section is 15 minutes. Also, since digestion was found to be as small as 5 per cent in some of the determinations, after cooking 15 minutes, it was deemed unwise to attempt quantitative determinations after cooking for less than 15 minutes. In Table VI are presented the results of digestion for 15 minutes and 2 hours, following 15 minutes, 30 minutes and 1 hour of cooking. The order of digestibility was practically the same in all of the individual

tests averaged (six for each cereal) namely; the Wheat Endosperm first; the "Whole Wheat" second, in all but one test; Precooked Oats third, in all but one; "Toasted Whole Wheat" fourth, in all but one. It is clear that the cereal preparation which contains no bran and no germ of the grain and is not precooked in any way is the most digestible in pepsin-hydrochloric acid. This is uniform for all the different cooking times.

TABLE VI

PER CENT PROTEIN MADE NON-PRECIPITABLE BY SODIUM TUNGSTATE BY

Digestion for — — —	15 min.		2 hrs.		
After cooking — — —	15 min.	30 min.	15 min.	30 min.	1 hr.
Wheat Endosperm	13.3	16.0	22.9	26.1	38.0
Precooked Oats	7.5	8.2	13.1	13.7	24.1
"Whole Wheat"	7.3	10.1	13.1	13.7	24.2
"Toasted Whole Wheat	7.2	6.2	13.6	12.8	19.0

The increase in digestibility to 15 minutes exposure after 30 minutes, as compared with 15 minutes, cooking is from 1 to 3 per cent in terms of nitrogen or protein rendered soluble. In relative terms the increase is from 10 per cent in the case of the precooked oats to 20 or 25 per cent in the case of the whole wheat. In the case of the toasted wheat the average digestibility is actually less after cooking 30 minutes than after cooking 15.

We have also in Table VI a direct comparison, after digestion for 2 hours, for the effects of cooking 15 minutes, 30 minutes and 1 hour. The increase in digestibility for 30 minutes as compared with 15 minutes varies from 1.6 per cent absolute in the case of the "Whole Wheat" and the Precooked Oats to 3.2 per cent in the case of the Wheat Endosperm. These percentages again are in terms of protein rendered soluble. The relative increase varies from 5 or 6 per cent in the case of the "Whole Wheat" and Precooked Oats to nearly 15 per cent in the case of the Wheat Endosperm. Again we find that the Toasted Wheat is less digestible after 30 minutes of cooking than after 15 minutes. The reason for this, as was noted under salivary digestion, is the tendency of this product to clump with longer cooking (see Tryptic Digestion, p. 104). Skin formation, which is so troublesome with the double boiler, does not take place in the active bubbling which prevails in a single boiler. Cooking for 1 hour increases the digestibility, as compared with cooking for 30 minutes, from 6 to 12 per cent in terms of proteins rendered soluble, or, in relative terms, from about 40 per cent in the case of the Wheat Endosperm to about 80 per cent in the case of the "Whole Wheat" and Precooked Oats.

The clumping, just noted, does not seem to interfere with the more prolonged digestion so much as it does with the shorter digestion. We shall see that the results of clumping in tryptic digestion are somewhat different. The order of digestibility after cooking from 30 minutes to 1 hour is the same as already noted for cooking 15 minutes. In fact, in 5 out of 6 tests, averaged in Table VI, the order of digestibility, after cooking 1 hour, is, Wheat Endosperm first, "Whole Wheat" second, Precooked Oats third, and "Toasted Whole Wheat" fourth.

The conclusions which may be safely inferred from the results of peptic digestion are as follows: Cooking for 30 minutes increases the digestibility over cooking 15 minutes in all of the cereals except the Toasted Wheat. The maximum relative increase which occurs in the case of the "Whole Wheat" is about 1/3; in the case of the Wheat Endosperm it is about 1/4; and in the other cereals less. Cooking for 1 hour increases the digestibility over cooking 15 minutes and over cooking 30 minutes of all of the cereals. The maximum relative increase by cooking 1 hour as compared with 15 minutes is in the neighborhood of 80 per cent in the case of the "Whole Wheat" and in the neighborhood of 60 per cent in the case of the Wheat Endosperm. The maximum relative increase after cooking 1 hour as compared with 30 minutes is in the neighborhood of 75 per cent for the "Whole Wheat" and about 45 per cent in the case of the Wheat Endosperm and the "Toasted Whole Wheat."

The proteins and other nitrogenous compounds of the cereal grains found in the bran and the germ are less digestible than the proteins found in the endosperm of the grain (Holmes, 1919). This has often been observed in the case of breads made from whole grain and from highly milled grain (Snyder, 1901-1905; Rubner, 1883, 1916; Food (War) Committee of Great Britain, 1918). This is probably the reason why the Wheat Endosperm, being entirely free of bran and wheat germ, is the most digestible of the four cereals studied. It also probably accounts for the positions of the other cereals in the order of digestibility. Nothing need be said here about the relative biological values of the proteins contained in the germ and endosperm.

C. Tryptic Digestion

The same general method was followed for studying the effects of trypsin as for studying the effect of pepsin. Five grams of each cereal were weighed out, placed in a small beaker, and boiled with 80 cc. of water for the requisite length of time. After cooling, sodium carbonate was added so as to make the mixture contain 0.3 per cent of this salt. The beakers were then placed in the incubator and 8 cc. of a 3 per cent trypsin solution were dumped simultaneously from test tubes into all the beakers. Digestion then continued for the requisite lengths of time at body

temperature, at the expiration of which digestion was stopped by simultaneously dumping sodium tungstate from test tubes into the beakers. Adjustment of the reaction to a different end point for each cereal and addition of an equal volume of 95 per cent alcohol was found necessary to produce complete precipitation. Filtration and determination of the nitrogen found in the water-clear filtrate then followed.

Partly for lack of time, and in order to concentrate attention upon the comparison between different times of cooking, and partly, also, because less error is involved in a longer period of digestion than in a short period, a two-hour period was selected uniformly for the different tests. Tests were made with four different times of cooking, namely, 5 minutes, 15 minutes, 30 minutes, and 1 hour. For reasons which will be explained presently, additional tests, after cooking 1 hour under a reflux condenser, were made. The results of four experiments with duplicate determinations for each are summarized in Table VII. The general order of digestibility for these tests is as follows; the Wheat Endosperm first, the "Whole Wheat" second, the "Toasted Whole Wheat" third, and Precooked Oats fourth. There is just one exception to this order, namely, that with 5 minutes cooking the precooked oats stands ahead of the whole wheats.

TABLE VII

AVERAGE PER CENT PROTEIN DIGESTED BY TRYPSIN IN 2 HRS. AFTER

Cooking —	— 5 min.	*	15 min.	30 min.	1 hr.	1 hr. with condenser
Wheat Endosperm	61.4	76.6	81.5	72.6	66.3	81.5
Precooked Oats	55.2	55.9	54.1	59.4	55.1	60.6
"Whole Wheat"	54.7	61.5	68.8	67.1	59.6	69.4
"Toasted Whole Wheat"	52.2	63.8	68.7	61.2	59.2	61.3
Wheat Endosperm Toasted	62.5					

* Digestion with diastase and trypsin together.

It is noteworthy that after cooking 15 minutes digestibility is greater than after cooking 30 minutes in all except one cereal, namely, the Precooked Oats. It is still more noteworthy that digestibility is reduced still further by cooking 1 hour as compared with cooking 15 minutes. This also applies to all of the cereals except the Precooked Oats. Digestibility, after cooking 15 minutes as compared with cooking 5 minutes, however, is greater in all of the cereals except the Precooked Oats.

It was quite obvious from direct inspection of the beakers that the difficulty after cooking the longer times in single boilers was the tendency

of the cereals to form lumps. The Precooked Oats, being of coarser grain, did not show this tendency so much as the other cereals. That this was the real cause is proved by the experience with the condenser. The cause of clumping is the too rapid evaporation of water when the single boiler is used and active boiling is kept up continuously. The simplest remedy is to provide an air condenser so that water driven off in the form of vapor will drip back into the cooking vessel as water. Small Erlenmeyer flasks were substituted for beakers, and into the neck of each flask was fitted a rubber stopper with an upright glass tube about 1/4 inch in diameter and about four feet in length. These tubes were clamped in position from a shelf directly above the hot plate on which the cooking was done. This arrangement permitted condensation of the vapors rapidly enough to prevent clumping of the cereals.

The effect on digestibility is shown by comparison of the last column in Table VII with the next to last. The digestibility of all of the cereals is improved. In all except the "Toasted Whole Wheat" the digestibility is raised to the same level, or higher, as attained after cooking only 15 minutes in the usual manner.

Attention may be called to the last figure in the table, namely, the effect of toasting on digestibility with trypsin. Samples of the Wheat Endosperm product were toasted in a baking oven until a light brown color was obtained, very similar to that which prevails in the "Toasted Whole Wheat." In contrast with the effects of toasting upon digestibility of starches and dextrins, we see that toasting does not affect digestibility of proteins; for the average figure, namely, 62.5 per cent obtained after toasting is substantially the same as obtained, 61.4 per cent, before toasting. Attention may be called also to the extra vertical line of figures shown in Table VII under "Cooking 5 minutes." These figures represent averages of two determinations made upon the digestibility of protein under trypsin acting simultaneously with diastase. Correction has been made for the effects of trypsin on the proteins of diastase itself.

The evidence from these few determinations is that trypsin acting simultaneously with a starch-splitting enzyme produces its proteolytic effect more rapidly than when acting alone. We shall see in a moment that trypsin reciprocally favors the action of diastase.

The conclusion under this section may be stated briefly as follows: Cooking for 15 minutes in a single boiler increases digestibility by trypsin over cooking 5 minutes in all cases except the Precooked Oats. The maximum relative increase in digestibility is in the neighborhood of 30 per

cent in the case of the Wheat Endosperm. Cooking in a single boiler for longer than 15 minutes decreases digestibility because of the formation of lumps produced by too rapid evaporation of water. This may be prevented by using a simple air condenser, thus restoring digestibility to its maximum obtained by 15 minutes of simple boiling. There is no advantage, however, in digestibility obtained by cooking up to 1 hour, even with a condenser, over cooking 15 minutes. Simple toasting does not increase the digestibility of the Wheat Endosperm under the action of trypsin as it does under the action of saliva. Simultaneous action of diastase with trypsin facilitates the proteolytic action of the latter ferment probably because the proteins are set free by simultaneous digestion of starch more rapidly than otherwise.

D. Action of Malt Diastase

In order to study the isolated effect of diastase uncomplicated by the action of any other enzyme, the diastase of malt was selected mainly because it is available commercially in purer form than any other diastase. Its action is, in general, similar to that of pancreatic diastase, but pancreatic diastase entirely free of trypsin is difficult to obtain.

The main consideration in studying the effects of another starch-splitting enzyme, in addition to the ptyalin of saliva, is that normally, in the intestine, a diastatic enzyme (amylase of the pancreatic juice) is active simultaneously with trypsin. It was desirable to determine whether this simultaneous action of trypsin and diastase would produce more rapid formation of sugar, particularly after a brief period of cooking, than action of diastase alone.

Only one complete set of experiments was carried through on this plan (Table VIII) but they are sufficient to prove quite conclusively, because the results are concordant, that the simultaneous action of the two enzymes is much more favorable than was obtained with diastase

Table VIII

Effect of Diastase on Starch of 5 Grams Cereal After Cooking 5 min.

	Per cent starch digested to sugar in 2 hrs.		Per cent starch rendered soluble in 2 hrs.	
	Diastase alone	Diastase with trypsin	Diastase alone	Diastase with trypsin
Wheat Endosperm	24.8	41.5	89.4	89.4
Precooked Oats	31.9	33.5	75.3	74.7
"Whole Wheat"	28.3	41.2	84.6	84.2
"Toasted Whole Wheat"	27.9	41.6	87.5	87.1

alone, or indeed with saliva alone. The largest effect shown in Table VIII is with the Wheat Endosperm, as would be expected from the fact, already demonstrated in previous sections, namely, that this cereal is the most digestible from the standpoint of its protein content. Rendering the proteins soluble by digestion obviously makes the starch more available to the diastatic enzyme.

The next largest effect occurs in the case of the "Toasted Whole Wheat," which is nearly the same as the effect with the "Whole Wheat," but the effect with Precooked Oats is very slight owing probably to its relatively slow digestibility under the action of trypsin. It should be noted that all of these tests were conducted on the cereals cooked only 5 minutes.

There is practically no effect of the simultaneous action of the two enzymes as compared with diastase alone when we consider the total sugar obtainable from the filtrate after hydrolysis. In other words, it would appear that the favorable action of trypsin has to do with the immediate formation of sugar rather than the formation of soluble dextrins.

Table VIII is already so brief that it need not be summarized further. The conclusion from this section of our study can be stated in very few words. Malt diastase transforms the starches and dextrins of the breakfast cereals into maltose very rapidly; much more rapidly, however, when trypsin is acting simultaneously with the diastase. Cooking 5 minutes is sufficient to insure digestion of the starch to the extent of 75 to 90 per cent in 2 hours.

Discussion

In her excellent study of the digestibility of different starches as affected by cooking, Day (1908) reviews the literature of starch chemistry and finds that three substances are present in raw starch grains, which are designated by her, according to the color they give with iodin, blue amylose, red amylose and rose amylose. Blue amylose constitutes 90 per cent or more of the inside of the cereal starch grains; it takes up water at 60 to 80°C and forms the sticky, colloidal substance known as starch paste, in which form it is easily digested. Long boiling up to 3 hours does not make it more digestible. Red amylose makes up the outer layer of starch grains. It swells at temperatures necessary for paste formation and breaks on boiling. In either case it does not hinder digestion. Rose amylose constitutes about 10 per cent of the inner starch. It is found only in cereal starches. It digests more slowly than blue or red amylose, but is converted slowly to blue amylose by cooking and this fact alone accounts for the greater digestibility of cereal starches by long cooking.

But there is a fourth substance found in cooked starch, designated as a reverted amylodextrin, by Syniewski, which is more difficult of solution than any of the amyloses in natural starch. It is found especially in the skin of starch paste and renders this much less digestible. If skin is permitted to form in the cooking of cereals the slight advantage gained by longer, over shorter, cooking, is thereby defeated.

These facts brought to light by Day doubtless acount for the results on the effects of cooking different lengths of time, as shown in this paper. Even the raw cereals, Wheat Endosperm and "Whole Wheat" whether digested for 5 minutes, 1 hour or 2 hours, did not show very much greater digestibility after boiling 15 minutes than after boiling only five minutes (Table II). The precooked cereals showed even less improvement. In these short cooking times the rose amylose was not changed to blue amylose. Comparing 30 minutes boiling with 1 hour, the digestibility was improved (Table IV) by the longer boiling about 50 per cent for the raw cereals, but not at all for the precooked. Skin formation was prevented in these experiments by stirring. The rose amylose must have been largely converted by 1 hour of active boiling and no reversion product was formed. Moreover, the cellulose cell walls in the raw cereals were broken down thereby liberating more starch to the action of the salivary amylase.

Our results with the sodium tungstate precipitation, however, reveal the interesting fact that the preliminary digestion to soluble dextrins (not to sugar) is improved to about the same degree (only 3 to 7 per cent) in raw and precooked cereals by boiling one hour over boiling 30 minutes. Since Langworthy and Deuel (1920) have shown that raw corn and wheat starches are completely digested in the human alimentary tract, we agree with Day that thorough salivation of cereal starches is probably much more important than long cooking, so far as starch digestion is concerned.

Only wheat and oats cereals not previously cooked to any extent have been compared in this series of experiments. The results indicate (second study p. 94) that the starch of Wheat Endosperm and of "Whole Wheat" both is more digestible than oats starch in the whole grain. This is in agreement with Woods and Snyder (1906) in their summary of many studies made at different experiment stations on digestibility of different cereals in the human alimentary tract. They find that the three cereals wheat, oats and corn, stand in the order named as to digestibility. There is more reason then for precooking, steaming or toasting, corn and oats than wheat.

When we consider the digestibility of protein in the cereal breakfast foods we find a different order of facts. The protein of wheat endosperm is more digestible than the protein of whole oats whether precooked or not. This applies to all different cooking times and periods of digestion tried. It is true of tryptic digestion as well as of peptic. The results also are in accord with all of the studies reported in the literature on the digestibility of white (endosperm) flour breads as compared with "whole wheat" or Graham breads. They indicate further that the low coefficient of digestibility of whole grain products is not due to greater elimination of metabolic nitrogen from the alimentary tract.

The improvement in digestibility by longer cooking (up to 1 hour) is greater for the protein than for the starch of the cereal breakfast foods. This applies particularly to the whole grain products. The reason doubtless lies in the destruction of cellulose walls especially of the bran. Clumping of the cereal which is so apt to occur in single boilers interferes greatly with this breakdown of the cells.

This study has not been concerned with the digestibility of the small amount of fat in the cereal breakfast foods nor with the optimum conditions for activity of the various enzymes employed. Every effort was made to have the conditions the same in simultaneous tests with the different cereals.

Summary and Conclusions

1. Six different cereal breakfast foods, all very widely used, have been compared as to their digestibility by salivary amylase, pepsin-HCl, trypsin and malt diastase.

2. Three different methods of study have been used and are described.

3. Only the results of the third method are given in detail. This method made use of sodium tungstate for precipitation of undigested residues. Clear filtrates were obtained and consequently satisfactory duplicate determinations of the stage of digestion reached. A dumping mechanism was employed for starting and stopping the digestions simultaneously for the different cereals.

4. The effects of different cooking times were closely studied.

5. The breakfast foods studied included three of the precooked variety and three previously uncooked.

6. Precooking affects favorably only the digestion of starch.

7. Cooking (boiling) improves the digestibility of the starch less than the digestibility of the protein.

8. Clumping of the cereal in open boiling retards digestion considerably and protein digestion more than starch digestion.

9. The simultaneous action of a proteolytic enzyme (trypsin) and a diastase produces much more rapid digestion of starch and protein than the diastase or trypsin acting alone.

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